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DEVELOPMENTAL ANATOMY OF THE FLESHY STORAGE ORGAN OF DAUCUS CAROTA[1]

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THE PRESENT STUDY of the anatomy of the fleshy, edible storage organ of the carrot plant (*Daucus carota* L.) describes, in different stages of development, the tissues making up this organ and interprets its gross morphological features in terms of histological details.

This investigation was completed when a paper by Havis (1939)[3] on the anatomy of the hypocotyl and root of the carrot appeared in press. Since Havis' article gives fewer ontogenetic details and emphasizes other points than the present study, a publication of the latter constitutes no duplication of material.

MATERIAL AND METHODS

Seedlings obtained by germinating carrot seed upon moistened moss in desiccators served for the study of the earliest stages. Larger plants were grown in a sandy soil in a greenhouse. The seed was of the variety Imperator.

The material, killed in a chrom-acetic-formalin solution (Rawlins, 1933), was imbedded in paraffin after being dehydrated with ethyl and butyl alcohols. The microtome sections were stained with the combination of tannic acid and iron chloride recommended by Foster (1934). Certain seedlings cleared in lactic acid were particularly useful in studying the transition region.

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[3] See "Literature Cited" for complete data on citations, which are referred to in the text by author and date of publication.

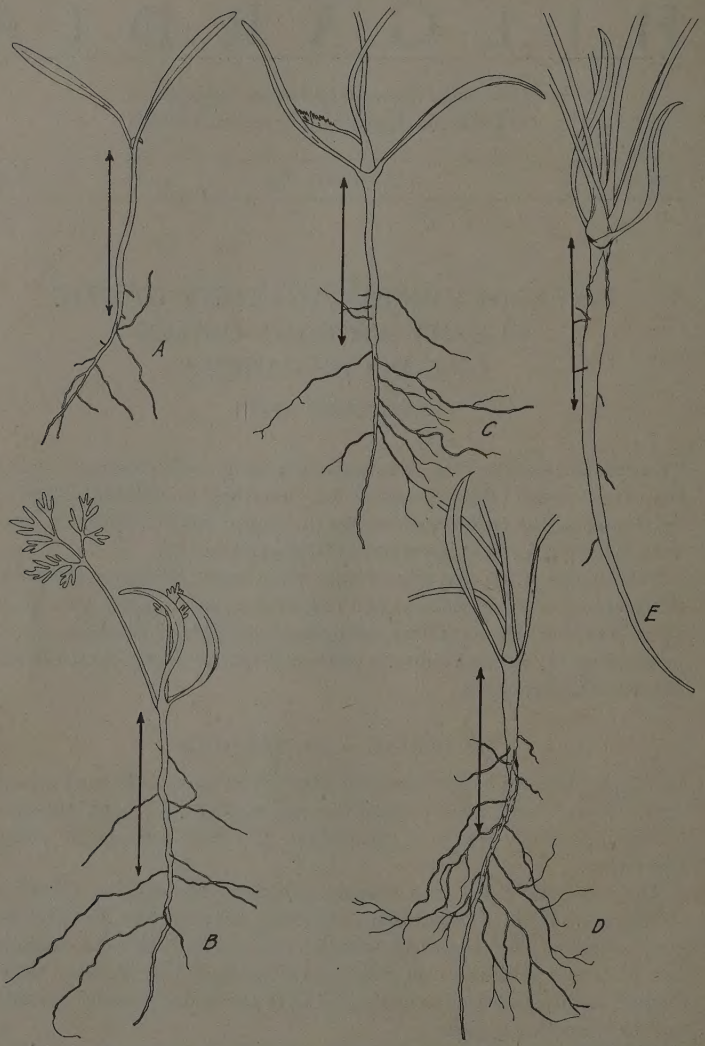

Fig. 1.—Carrot plants in successive stages of development collected the following number of days after the seed had been sown: *A*, 11; *B*, 18; *C*, 25; *D*, 32; *E*, 39. The seedlings were up in four days. The larger foliage leaves have been removed in *C–E;* the lateral roots in *E;* the thin portion of the taproot in all plants. *D* shows the beginning of rupture of the cortex. In *E*, the root region has no cortex; the hypocotyl shows fragments of it. The arrows indicate the approximate length of the hypocotyl, which had ceased to elongate in these plants. (All × 1.)

Figures 3; 4; 5, *A–D;* 8, *A, C,* and *D;* 9; and 12, *A;* and plates 1 and 2 were prepared from sections of the seedlings that were grown in desiccators. For all other illustrations in the paper, the greenhouse plants were used.

EXTERNAL MORPHOLOGY

Before the foliage leaves appear, the young carrot plant shows a rather clear demarcation between the taproot and the hypocotyl. The latter bears no lateral roots at first and is thicker than the root. The epidermis of the root has root hairs, structures that do not appear on the hypocotyl. Sometimes the change in thickness from the hypocotyl to the root is very abrupt (fig. 1, *C*). Above, the hypocotyl gradually merges with the cotyledons whose bases are united (fig. 1, *C*, and plate 7, *A*.) When the axis bearing the rosette of leaves develops from the plumule enclosed between the cotyledons, the bases of the latter are greatly stretched (plate 7, *B*).

The lateral roots are arranged in four longitudinal rows. They appear first at the base of the root—that is, just below the hypocotyl—and then develop toward the root apex. Later they also arise on the hypocotyl, first at its base (fig. 1, *A*), then acropetally toward the cotyledons (figs. 1, *B–E*, and 2). The lateral roots remain thin.

With the development of the lateral roots and with the increase in thickness of the hypocotyl and the taproot, the external distinction between the two regions is gradually effaced until they merge into one elongated tapering structure (figs. 1, *E*, and 2). In the mature state the hypocotyl makes up about 1 inch of the upper part of the fleshy organ, although its length varies somewhat with the depth of planting of the seed and certain other environmental conditions. The lower part of the storage organ is derived from the taproot (figs. 1 and 2).

The increase in thickness of the root and the hypocotyl results in the loss of the cortex. The rupture of this layer occurs first at the base of the root, then advances toward the apex of the root and acropetally in the hypocotyl (fig. 1, *D* and *E*). Thus the deterioration of the cortex proceeds in the same direction as the development of the lateral roots. In older storage organs the cortex is entirely absent (fig. 2, *B–D*) and the surface is covered with a periderm which bears horizontal grooves at the base of each lateral root (fig. 2, *D*).

The characteristic orange color appears after the loss of the cortex, approximately at the stage shown in figure 1, *E*. It is evident first in the root portion of the storage organ and later becomes evident in the hypocotyl.

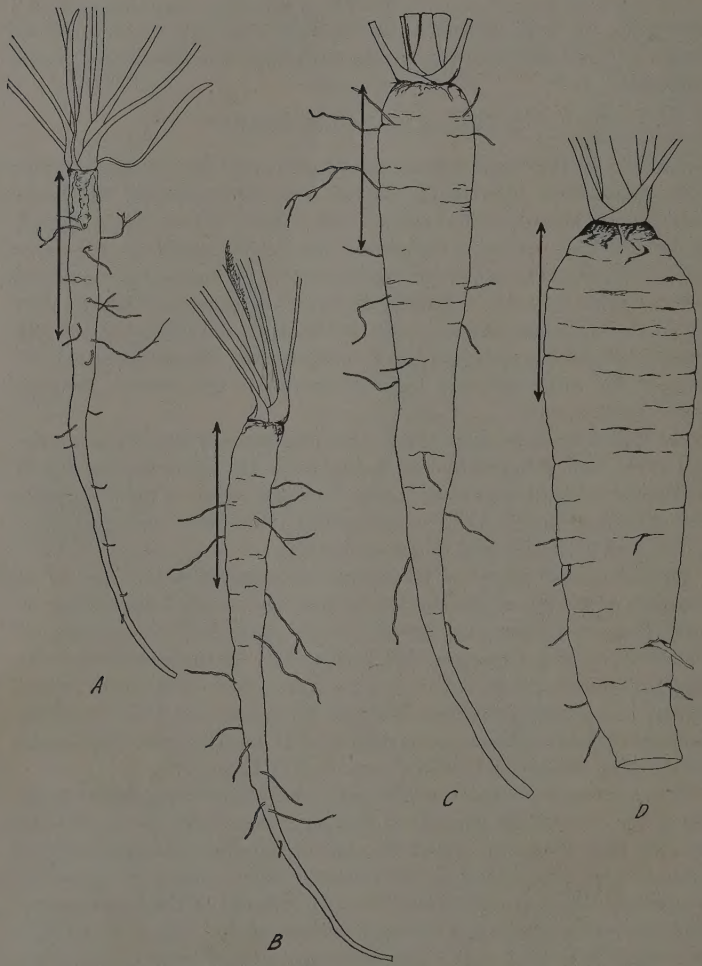

Fig. 2.—Carrot plants in successive stages of development, showing increase in size of the fleshy hypocotyl and root. The plants were collected the following number of days after the seed had been sown: *A*, 46; *B*, 53; *C*, 60; *D*, 67. The lateral roots, the thin portion of the taproot, and the leaves have been removed in all plants. The cotyledons and fragments of the cortex of the hypocotyl are present in *A*. In the other plants the cotyledons and the cortex are absent. The arrows indicate the approximate length of the hypocotyl. (All × 1.)

ONTOGENY OF THE PRIMARY ROOT

The root tip of the seedling is composed of four regions: the meristematic stele, the meristematic cortex, the meristematic epidermis, and the rootcap (fig. 3, *A*, *B*, and *F*). Though a detailed study of the apical meristems was not intended in this investigation, an examination of several root tips of seedlings has indicated that the apex lacks a clear-cut differentiation into the initials of the stele, the cortex, and the epidermis. The cells at the apex of the central cylinder appear to be continuous with the longitudinal rows of the rootcap cells (fig. 3, *A*), as if the stele and the central portion of the rootcap were derived from the same initials. Transverse sections give a similar picture. The dividing cell in the stele in figure 3, *F*, is apparently cutting off one cell toward the stele, the other (fig. 3, *E*) toward the rootcap. Judging from the presence of starch grains, the section in figure 3, *E*, is part of the rootcap. The cortex and the epidermis have a common origin with the peripheral portion of the rootcap. Nearer the center of the cap certain initials give rise to cortical and rootcap cells by alternating anticlinal and obliquely periclinal divisions. On the periphery the rootcap cells are formed by periclinal divisions in the epidermis (fig. 3, *A*).

The rootcap characteristically contains starch grains (fig. 3, *C* and *D*), which become evident immediately adjacent to the apical initials (fig. 3, *E*). The dead peripheral rootcap cells, on the other hand, lack starch and have somewhat thick walls (fig. 3, *C*).

The cortex is increased in thickness, usually from two to four or five cells, by periclinal divisions of the innermost layer of cells (figs. 3, *A*, *B*, and *F*, and 4, *C*, *E*). The first of these divisions occurs just behind the apical initials (fig. 3, *A* and *F*). After the last division the layer next to the stele differentiates into an endodermis (fig. 5, *B*). The Casparian strips appear in the endodermal cells after the differentiation of the first sieve tubes and shortly before the maturation of the first vessels. The strips, which are rather narrow, occur on the radial and the transverse walls near the inner tangential wall.

Whereas the youngest cortical cells are approximately isodiametric (fig. 3, *F*), the older ones are tangentially elongated (figs. 4, *E*, and 5, *A*; plates 1 and 2, *A*). At first the epidermal cells show radial elongation (fig. 5, *A*, and plate 1); later their tangential diameters increase somewhat (plate 2). (The poor fixation usually obtained in the elongating region of the root has caused the distortion of the cell shape in plate 2, *B*.)

The cortical intercellular spaces first appear between the second and the third layers from the epidermis (plate 1). Later they spread inward

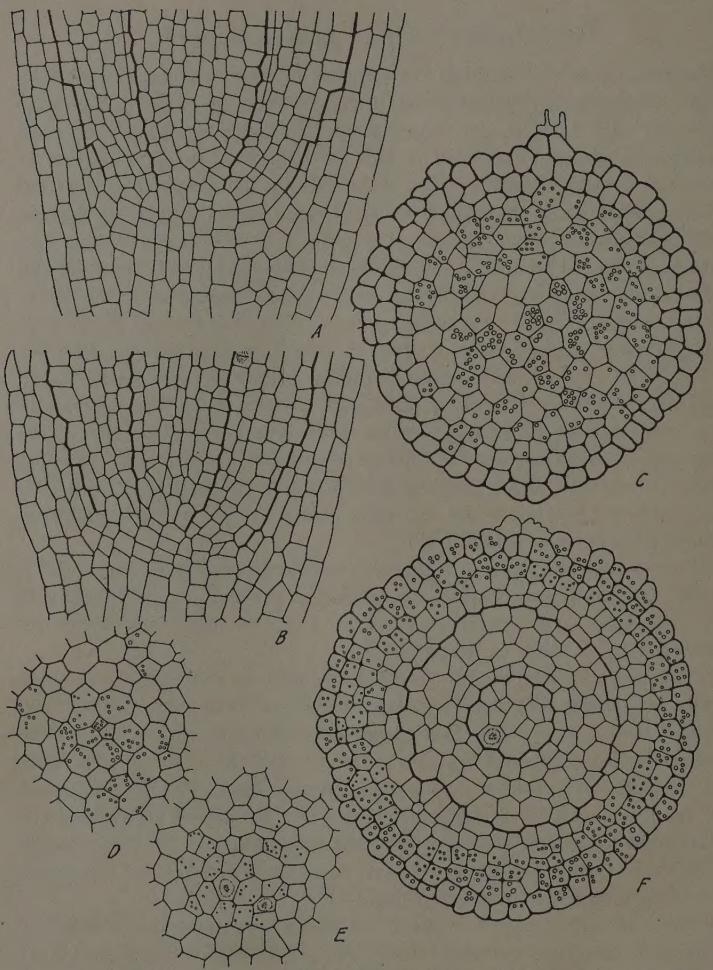

Fig. 3.—Structure of the root apex of a seedling. *A* and *B*, Longitudinal sections showing the region of apical initials. *A* is the median section; *B* is 10 microns away from *A*. The heavy lines indicate the limits of the central cylinder and the cortex jointly with the epidermis. Rootcap cells appear right and left and below in each drawing. *C–F*, Successive transverse sections of the root apex. *D* and *E* show only the central portions of the sections. *C–E* represent the rootcap region; *F*, the region of apical initials. The small circles in *C–F* represent starch grains. The heavy lines in *F* delimit the stele, the cortex, jointly with the epidermis, and the rootcap. (All × 372.)

(plate 2) and outward in the cortex, but do not develop between the epidermal and the subepidermal layers (fig. 5, *B*, and plate 3, *A*).

The stele is clearly set off from the cortex just behind the terminal

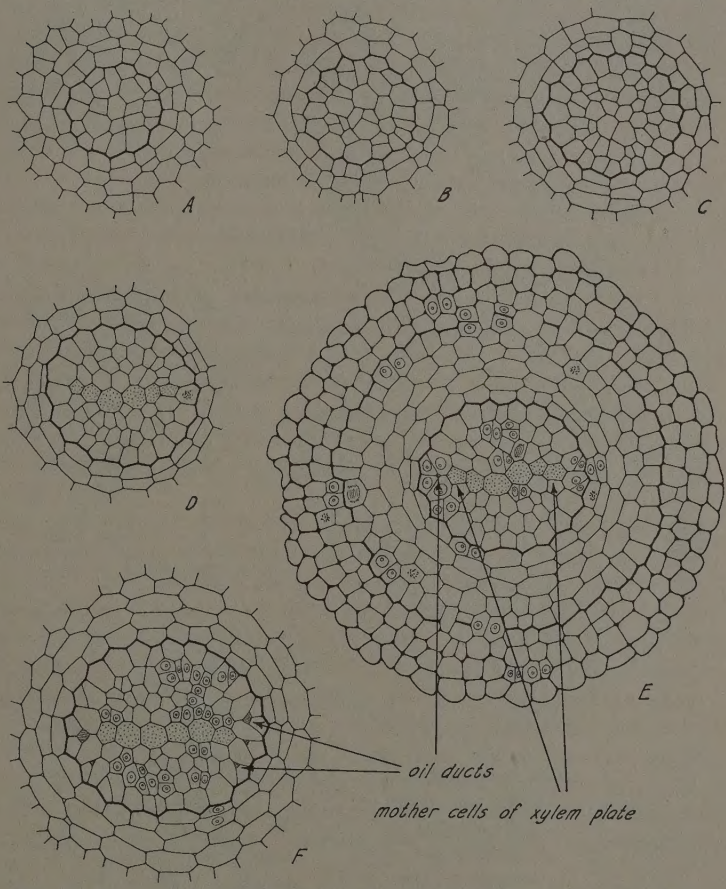

Fig. 4.—Successive transverse sections of root apex continuing the series shown in figure 3, *C–F*. *A–D*, and *F* show only the central portions of the sections. The oil ducts in *E* and *F* are in the pericycle. The heavy lines indicate the limits of the stele and of the cortex jointly with the epidermis. (All × 372.)

meristem (fig. 3, *A* and *F*). Within the stele the first region to become individualized is the pericycle (fig. 4, *C*). In longitudinal sections this layer may appear to be entirely independent; but in transverse sections

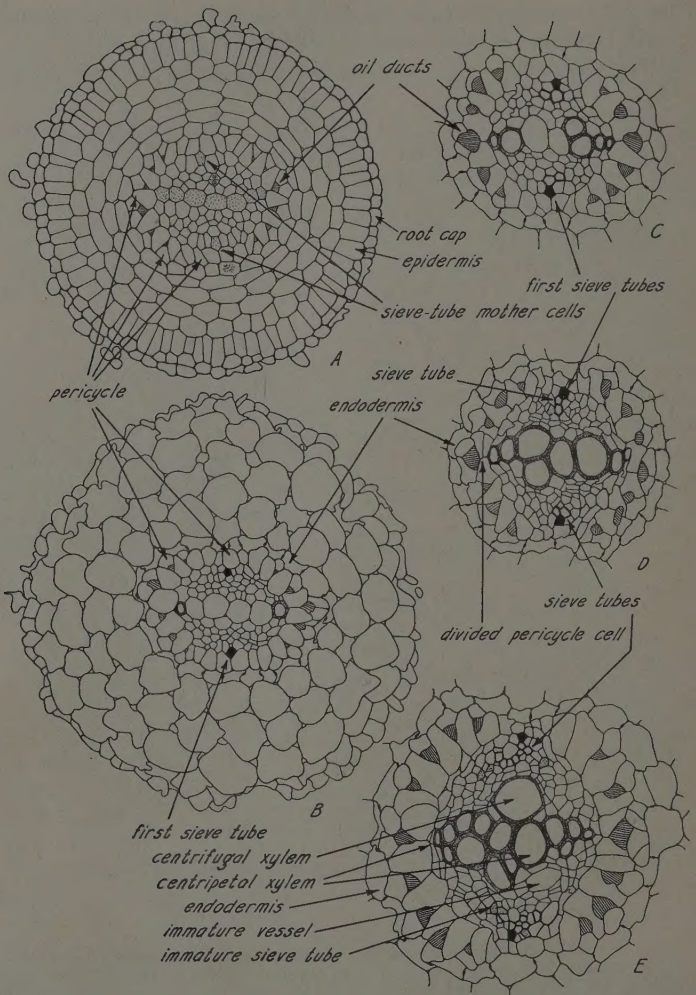

Fig. 5.—Transverse sections of young taproots in different stages of development. *C–E* depict only the central portions of the sections. *A–C* complete the series of transverse sections shown in figures 3 and 4. *D* and *E* were taken from older plants, the one used in *E* being comparable with the plant in figure 1, *A*. Some of the immature vascular elements are indicated by stippling; the pericyclic oil ducts by hatching. The first two sieve tubes are shown in black. (All × 256.)

it is not clearly outlined at first (figs. 3, *F*, and 4, *A*, *B*). It becomes distinct about 20 microns from the apex of the root (fig. 4, *C*). Twenty microns farther the pericycle undergoes a series of oblique longitudinal divisions (fig. 4, *E*). The resulting cells form a characteristic pattern, with intercellular spaces—the pericyclic oil ducts—appearing at the junction of the oblique and the radial walls (figs. 4, *E* and *F*, 5, *A* and *B*; and plates 1, 2, and 3). Oil ducts traverse the pericycle horizontally as well as vertically (plate 9, *A*) and continue into the cotyledons (plate 8).

After the delimitation of the pericycle, the cells in the interior of the stele continue to divide (fig. 4, *C*). About 30 microns from the apex, a median row of cells becomes conspicuous because of the enlargement and vacuolation of its components. These are the vessel mother cells of the future xylem plate (fig. 4, *D*, and plate 1, *A*). Enlargement and vacuolation begin in the middle of the plate, then spread toward the cells next to the pericycle (plates 1 and 2). The outermost cells of the plate are, however, the first to mature into xylem elements (fig. 5, *B*). They do not attain as large a size as the cells in the center. Usually the xylem plate is complete from pericycle to pericycle as soon as it becomes evident. It does not, however, remain a one-celled row, since other laterally adjacent cells also enlarge, vacuolate, and later differentiate into xylem elements (fig. 5, *C* and *D*).

The cells enclosed between the primordial xylem plate and the pericycle on both sides of the plate continue to divide in all planes (fig. 4, *E* and *F*). About 200 microns from the apex of the root, the mother cells of the first two sieve tubes are perceptible in the median peripheral positions in these two areas (fig. 5, *A*, and plate 1, *A*); and the organization of the stele is definitely established. This region now contains a primordial diarch xylem plate reaching from pericycle to pericycle; two sieve-tube mother cells located opposite each other and next to the pericycle, in a plane at an angle of 90° to the xylem plate; and a pericycle, which is one-layered opposite the future protophloem but double-layered elsewhere. The pericycle has oil ducts arranged in two arcs, each with the central duct usually located opposite one of the future protoxylem points.

Below the region where the vascular elements mature, the longitudinal cell divisions are more numerous in the stele than in the cortex. The cells of the latter become, therefore, considerably larger, in transverse sections, than the stelar components. Within the stele itself the pericycle and the xylem cells attain larger transverse diameters than the cells in the phloem region (fig. 5, *A* and *B*, and plates 1 and 2).

The two sieve-tube mother cells are the first among the vascular ele-

ments to reach maturity. They elongate and lose their nuclei (fig. 12, *A*); their cytoplasm vacuolates and becomes definitely parietal. About 300 microns from the apex of the root the two protophloem sieve tubes are fully differentiated. Although these elements have plastids, their contents are otherwise as clear as those of the pericyclic oil ducts (fig. 12, *A*, and plate 1, *B*). Their transverse walls bear sieve plates, and the longitudinal ones show thickening layers—the *nacré walls*. (See review by Esau, 1939.) As the elements further elongate, these walls become thinner. In figure 12, *A*, only two cells show the nacré walls; the other mature elements have rather thin ones.

Since their mother cells rarely divide by longitudinal walls, the two protophloem sieve tubes have usually no companion cells. The series of cells shown in figure 12, *A*, could be followed almost to the apex of the root; but among the twenty-four cells in the series, only one was divided longitudinally. At higher levels several more sieve tubes, also lacking companion cells, differentiate centripetally with respect to the first two phloem elements (fig. 5, *C* and *D*). Shortly before the end of the primary growth, sieve tubes with companion cells appear.

The first two protoxylem elements mature after the first two sieve tubes. In the same seedlings where the first phloem cells have matured about 300 microns from the terminal meristem, the vessels begin to develop secondary walls at the 1-mm level and are fully differentiated 2 millimeters from the apex—slightly below the root-hair region. Like the first sieve tubes, the first xylem elements lie next to the pericycle (fig. 5, *B*). The points in a cross section of a stele at which xylem and phloem elements first mature are sometimes conveniently called the *protoxylem* and the *protophloem* poles, respectively.

The subsequent xylem elements develop centripetally with respect to the first two (fig. 5, *C, D*, and plate 3, *B*). After the differentiation of the centripetal xylem, a few more elements appear centrifugally in relation to the diarch xylem plate (fig. 5, *E*). These are not yet derived from the cambium but lie in line with the future secondary vessels. As is usual with the primary xylem of roots, the diarch plate consists of annular, spiral, scalariform, and reticulate vessels.

The first xylem having annular and spiral elements and lying near the pericycle is commonly called the *protoxylem;* the elements appearing later and in the center of the xylem plate, the *metaxylem*. The primary centrifugal xylem is also regarded as metaxylem. In conformity with the xylem, the phloem appearing first and nearest the pericycle is termed *protophloem*. It is followed by the *metaphloem* differentiating centripetally with respect to the protophloem.

With the development of the centrifugal xylem, the primary growth of the root is completed. Figure 5, *E*, depicts the structure of the stele and the endodermis at the beginning of the secondary growth. The cells between the xylem and the phloem show the first tangential cambial divisions. The pericycle cells opposite the protoxylem poles have split off daughter cells that later will give rise to cambial cells.

The first protoxylem elements and the first sieve tubes are obliterated at the beginning of the secondary growth—a process that occurs somewhat earlier in the hypocotyl than in the root.

The seedling completes the primary development of the base of the root and of the hypocotyl just before the leaves emerge from the plumule. The plant depicted in figure 1, *A*, shows early stages of secondary growth.

THE TRANSITION REGION

The transition from the root to the stem structure is very gradual and occurs through most of the hypocotyl. The branching of the primary xylem plate in the formation of the cotyledonary traces[4] is, however, rather abrupt (plate 8); and the change from the exarch to the endarch xylem occurs in the cotyledons. The hypocotyl therefore resembles the root rather than the stem in its internal structure. As was earlier pointed out, the hypocotyl resembles the root also in external morphology.

The beginning of transition is indicated in the vascular tissues by the change in the arrangement of the elements. In the root the first sieve tubes form compact clusters; in the transition region they are somewhat dispersed among parenchyma cells. In the root the succeeding sieve tubes develop centripetally with respect to the first (fig. 5, *C–E*); in the hypocotyl they appear laterally from the protophloem poles (figs. 6, *A–C*, and 8, *A*). In the root the first protophloem sieve tubes lie next to the pericycle; in the hypocotyl they are separated by parenchyma from the pericycle (fig. 6, *C*).

In later stages of the primary development and during secondary growth in transition region, the lateral spread of the oldest phloem becomes less obvious because numerous sieve tubes differentiate centripetally with regard to the first (fig. 8, *B*).

The xylem of the hypocotyl differs from that of the root in the occurrence of parenchyma between the protoxylem and the pericycle in the former (fig. 6 and plate 3). This characteristic appears at the base of the hypocotyl. Somewhat higher, the order of vessel development is also

[4] The term *trace* is applied here to each individual bundle that connects the leaf with the stem and hypocotyl.

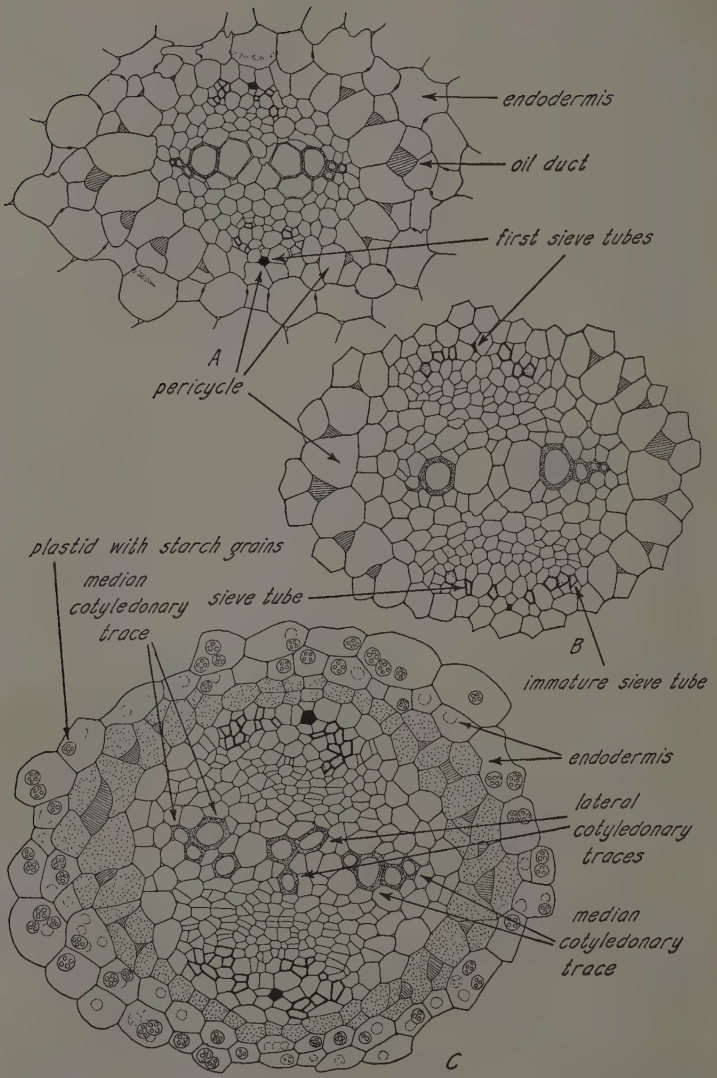

Fig. 6.—Successive transverse sections of a hypocotyl, showing the beginning of transition from root to stem structure. *A* is nearest the root base; *C*, farthest away. Details are as in figure 5 except that the pericycle cells are stippled in *C*. The plant was sampled four days after the seed had been sown. (*A*, × 269; *B* and *C*, × 400.)

modified. At first the xylem differentiates centripetally from two points as in the root. Then some elements mature in the middle of the plate so that the latter appears broken up into three bundles (fig. 6, *C*). Some of the cells separating the bundles are parenchyma; others are vessel mother cells. When the latter mature, the xylem appears as one strand (fig. 8, *A* and *B*).

The successively higher levels of the hypocotyl show an increasing parenchymatization of the xylem. Whereas in the root the xylem plate consists of vessels only (fig. 5, *E*), in the upper transition region it encloses considerable parenchyma (fig. 8, *B*).

Below the insertion of the cotyledons the xylem is represented by two large and three small groups of vessels (fig. 7, *C*). The large groups are the median cotyledonary traces; the small ones contain the first xylem elements of the lateral cotyledonary traces. One of these small groups gives, at higher levels, two branches, so that altogether four lateral traces are formed, two for each cotyledon (fig. 7, *D*, and plate 7). The median traces of the cotyledons are continuous with the protoxylem poles of the root, showing a centripetal order of differentiation for some distance within the cotyledons. The lateral traces are branches of the central portion of the xylem plate. In the early stages of development this portion appears, in the lower hypocotyl, as a separate bundle (fig. 6, *C*). The lateral traces show centrifugal xylem differentiation throughout their extent. At successively higher levels all these traces differentiate somewhat more obliquely and farther away from the center of the axis, where cells mature into the parenchyma of the pith (fig. 7, *C*, and plate 8).

The phloem also develops at an increasingly greater distance from the center and, at the same time, continues to spread laterally from the protophloem poles. Just below the level where the xylem forms the cotyledonary traces, the first two sieve tubes disappear as such (fig. 7, *C*). However, these elements form connections with the sieve tubes differentiating to the right and to the left of them (fig. 7, *A* and *B*).

Whereas the xylem differentiates in six bundles at the base of the cotyledons, the phloem forms eight strands. The median traces of the cotyledons have each two strands of phloem flanking the xylem on two sides; the lateral traces are collateral bundles, each with one strand of xylem and one of phloem (fig. 7, *D*, and plate 7).

In the upper hypocotyl the pericycle does not form a continuous layer as in the root. Like the vascular elements, the oil ducts differentiate farther away from the center of the axis and also somewhat obliquely. Moreover, their number is reduced so that two or three ducts below are

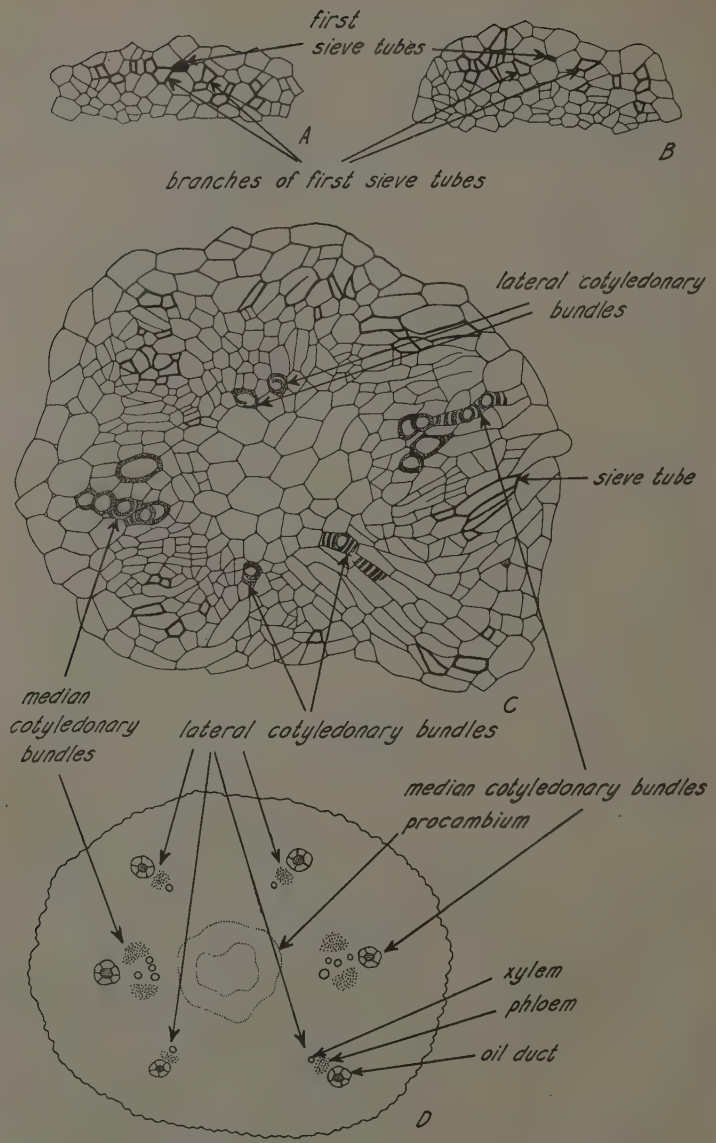

Fig. 7.—Successive transverse sections of the hypocotyl of the same plant as in figure 6. *A*, *B*, Details of branching of phloem in the transition region. *C*, Reorientation of the vascular tissues in the formation of the cotyledonary traces. *D*, Fused bases of cotyledons enclosing the procambium cylinder of the plumule. Compare with plates 6 and 7. (*A–C*, × 400; *D*, × 134.)

connected with one above. At the base of the cotyledons two groups of three ducts differentiate (fig. 7, *D*) instead of two groups of six to nine ducts as in the root and lower hypocotyl (figs. 5 and 6). These ducts occur on the abaxial sides of the cotyledonary bundles (fig. 7, *D*, and plate 7, *A*). As seen in transverse sections the secretory cells lining the ducts are more numerous in the cotyledons than in the root. In the latter, three or four cells surround the oil cavity (fig. 5); in the cotyledons, five or six (fig. 7, *D*). In the foliage leaves, the structure of the oil ducts is the same as in the cotyledons. Since the cells between the ducts do not differ from other parenchyma cells surrounding the cotyledonary bundles, the pericycle appears to consist of the individual oil ducts with their secretory cells (plate 7, *A*).

In contrast to the lower hypocotyl (fig. 6, *A*) and the root (plate 3, *B*), the endodermis of the upper hypocotyl is characterized by starch accumulation (fig. 6, *C*, and plate 3, *A*). The lowermost starch-containing endodermal cells also have Casparian strips, structures that do not differentiate at the higher levels. Within the cotyledons no specialized endodermis is evident (plate 7, *A*).

Although starch appears also in the cortex of the hypocotyl, it is less prominent there than in the endodermis (plate 3, *A*). The epidermis of the upper hypocotyl has stomata.

The primary vascular tissues of the root are continuous with the traces of the cotyledons. The foliage leaves differentiate after the initiation of secondary growth in the root, forming direct connection with the products of the cambium and perhaps also with the small amount of centrifugal primary xylem.

At first the vascular tissues of the cotyledons appear much more prominent than those of the foliage leaves. Later, because of the secondary growth, the leaf traces develop to a much larger size than the entirely primary cotyledonary strands (plates 6 and 7).

Above the insertion of the cotyledons in very young seedlings, the procambium of the foliage leaves forms an uneven ring within the circle of the cotyledonary bundles (fig. 7, *D*). At lower levels, where the traces of the cotyledons appear nearer the center of the axis, this procambium and later the leaf traces occur in strands among the cotyledonary traces (plate 6, *B*). Still lower the cotyledonary vascular supply appears imbedded in the xylem of the leaf traces (plate 6, *A*).

Two of the lateral bundles of the cotyledons become discrete near the center of the hypocotyl (fig. 7, *C*, below, and plate 6, *B*, left). The other two are united for some distance, then separate (fig. 7, *C*, above, and plate 6, *B*, right). The pair of separate lateral cotyledonary strands

occurs among the traces of the second foliage leaf; the united pair between those of the first leaf (plate 6, *B*).

ORIGIN OF THE LATERAL ROOTS

In the primary body the lateral roots are initiated in the pericycle to the right and to the left of the protophloem poles, making an angle of about

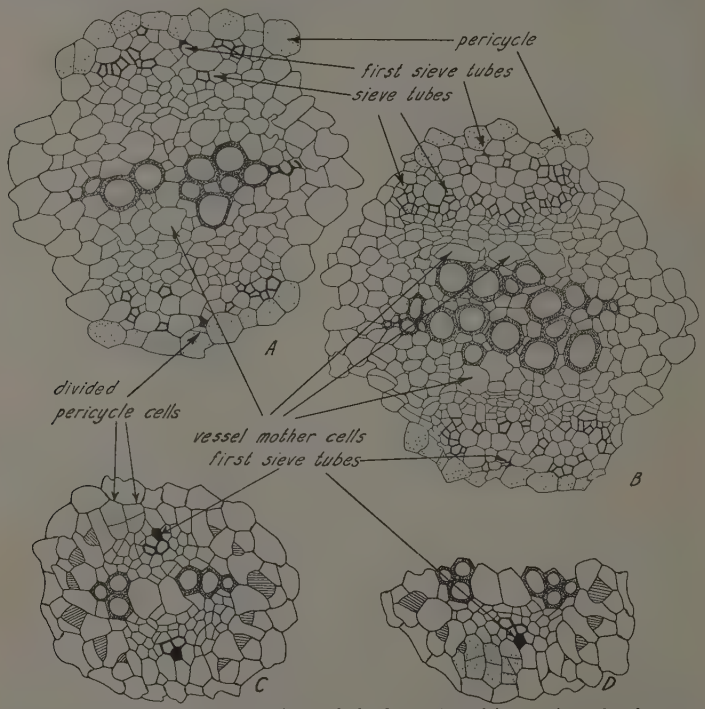

Fig. 8.—*A*, *B*, Transverse sections of the lower transition region, showing two stages of development of vascular tissues. *B* was taken from the lower hypocotyl of a plant similar in size to that in figure 1, *A*; *A*, from a younger plant. *C*, *D*, Transverse sections of a root of a seedling, showing initiation of lateral roots. In *C* two pericycle cells have divided by periclinal walls; in *D* three cells, one of which is a secretory cell of an oil duct. In *A* and *B*, all pericycle cells shown are stippled; in *C* and *D* only those that were concerned with lateral-root formation and showed very dense protoplasts. Other details as in figure 5. (*A*, ×421; *B–D*, ×283.)

45° with the xylem plate. There are, accordingly, four points of origin of lateral roots, two on each side of each phloem strand.

As seen in transverse sections, one or two pericycle cells to the right

or left from the first sieve tube undergo periclinal divisions (fig. 8, *C*, two of the stippled cells). Soon the secretory cells of the nearest oil duct undergo similar divisions (fig. 8, *D*); then those of the ducts that are farther removed. The divisions may spread to the median oil duct lying opposite the nearest protoxylem pole, the oil ducts being obliterated in the process (fig. 10, *A*). The cells participating in the divisions have densely staining protoplasts.

In longitudinal sections also, divisions are initiated in two or three cells and then spread to the adjacent ones. At first the cells divide transversely; then they elongate radially and divide periclinally (fig. 9, *A* and *B*); later, more periclinal and anticlinal divisions occur (fig. 9, *C*).

The endodermis remains intact until the lateral emerges on the surface of the main root. It shows dense protoplasts over the area of dividing pericycle cells, eventually forms anticlinal walls, and thus keeps pace with the growth of lateral root (fig. 9, *B* and *C*). The first new walls in the endodermis sometimes develop Casparian strips, structures not formed in the later divisions. The parenchyma of the cortex is destroyed in front of the advancing primordial root (fig. 9, *C*).

Before the lateral root emerges on the surface of the main root, the derivatives of the endodermis become stretched—a process most pronounced at the base of the lateral organ (fig. 10, *A*, above), where eventually the endodermal cells are torn apart. Although the emerging lateral carries the upper portion of the endodermis to the surface, these cells soon die off. They may begin to disintegrate while the lateral is still imbedded in the cortex of the main root (fig. 10, *A*, cells indicated by cross hatching).

The main part of the lateral root consists of the derivatives of the pericyclic cells. These are organized into a stele, a cortex, and a rootcap before the root emerges on the surface (fig. 10, *A*). As in the taproot of the seedling, the epidermis and the cortex appear to merge with the rootcap. The stele, which is clearly outlined, seems independent of the other root regions (fig. 10, *A*). As in the taproot, the cortex increases in thickness by periclinal divisions (fig. 10, *A*); and the endodermis differentiates in the last and innermost products of these divisions. The Casparian strips are evident before the lateral root breaks through to the surface. The new endodermis is connected with the endodermis of the main root through cells at the base of the lateral root. These cells develop Casparian strips before the endodermis develops in the lateral root proper (fig. 10, *A*, upper left).

The vascular tissues appear in the lateral after it has passed through the cortex of the mother root. The young root forms a connection, first

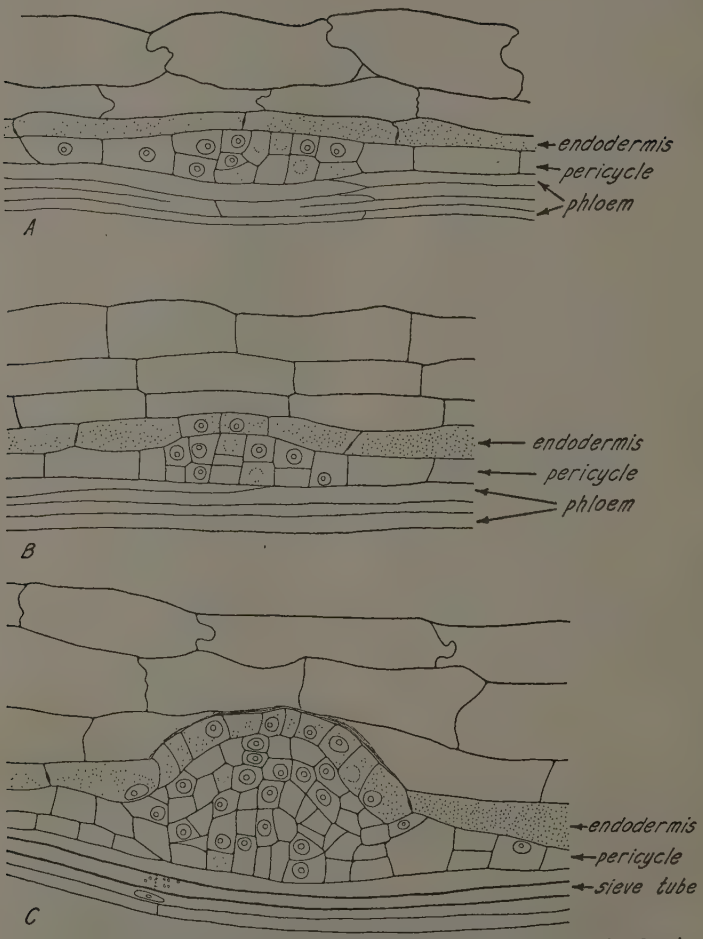

Fig. 9.—Three successive stages of development of a lateral root as seen in a longitudinal section of a young taproot. (All × 421.)

with one side of the primary xylem plate of the main root and with one phloem strand; later, when secondary growth occurs, also with the other phloem strand. Such is the orientation of the diarch xylem plate in the lateral that a line connecting the two protoxylem poles is more or less parallel with the long axis of the main root.

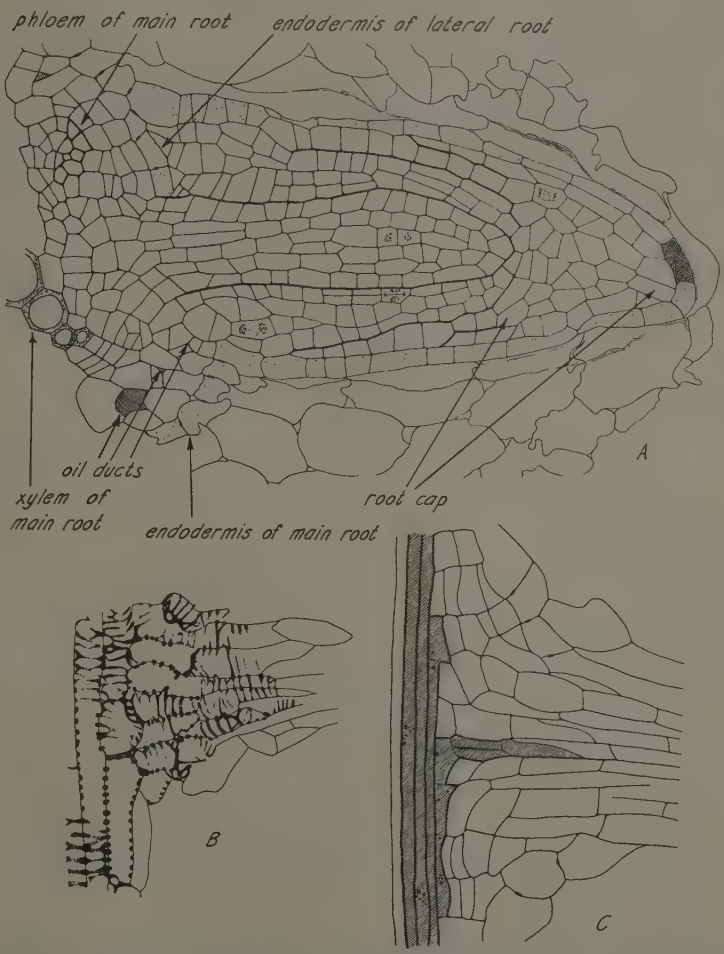

Fig. 10.—*A*, Lateral root just before emerging on the surface of the main root. A small amount of vascular tissues of the main root is seen in transverse section, at the left. The heavy lines indicate the probable limits of the plerome, the periblem, and the dermatogen. *B*, Xylem, and *C*, phloem, at the point of connection between the main and lateral roots. In *B* and *C* the vascular elements of the main root extend vertically to the left; those of the lateral roots are arranged horizontally to the right. The sieve tubes in *C* are indicated by hatching. (All × 380.)

The connection between the vascular tissues of the main and the lateral roots is formed through cells of pericyclic origin lying outside the protophloem and protoxylem of the main root. These are parenchyma cells of short diameters arranged like the derivatives of a cambium (fig. 10, *A* and *C*). Near the xylem they differentiate into scalariform tracheids and vessel elements (fig. 10, *B*), near the phloem, into sieve tubes (fig. 10, *C*) with which companion cells are associated.

Lateral roots continue to arise in the fleshy root and hypocotyl even after the cortex sloughs. At this time the lateral organs usually originate at the base of the older lateral roots from cells of pericyclic origin. They form vascular connections with the xylem and phloem of the main and the older lateral roots.

SECONDARY GROWTH IN THE ROOT AND THE HYPOCOTYL

The enlarged underground storage organ of the carrot plant is developed largely by secondary growth from the vascular cambium. As is usual in roots with secondary tissues, the periclinal cambial divisions first become evident between the xylem and phloem. Later they occur in the parenchyma outside the protoxylem, so that a complete cylinder of cambium is formed.

In the root region, where the protoxylem lies next to the pericycle, the cells of the latter divide before the secondary growth is initiated (fig. 5, *D*). Thus at the end of the primary growth some parenchyma occurs between the protoxylem and the secretory cells of the oil ducts (fig. 5, *E*). In the hypocotyl the conducting elements of the protoxylem do not differentiate next to the pericycle (figs. 6 and 8, *A* and *B*).

The amount of secondary vascular tissues produced by the cambium is greater in the upper than in the lower portion of the storage organ (plates 4 and 5). On the other hand, in the root the vessels tend to have larger diameters and are more compactly arranged than in the hypocotyl (plates 4 and 5). The change from one cell pattern to the other is, of course, gradual.

Except in the earlier stages of secondary growth (plate 4, *A*), the secondary xylem contains a large proportion of parenchyma (plates 5, *A*, and 11). In the hypocotyl considerable parenchyma occurs outside the protoxylem poles (plates 4, *A*, and 5, *A*). These islands of parenchyma cells are the first products of the cambium in this region. Later the cambium produces xylem and phloem, and the parenchyma becomes imbedded between the primary and secondary xylem. At the same time the vascular cylinder becomes continuous (plate 5, *A*). On the inside of the

primary phloem the cambium forms xylem as soon as the meristem begins to function (plate 4, *A*). Although secondary in origin, the vessel elements are scalariform and reticulate.

Besides the parenchyma of the longitudinal system, ray parenchyma occurs in the xylem. The rays, though not conspicuous in transverse sections (plate 11), are evident in longitudinal ones (plate 13). Tangential section through the cambium show the striking contrast between the ray and the fusiform initials (plate 9, *B*).

During the later stages of the secondary development, the parenchyma cells of the primary xylem enlarge and occasionally divide (plates 5, *A*, and 13, center). As a result of this growth the primary vessels become widely separated from each other and from the secondary vessels. During this process, there is also some crushing of the primary xylem. The dilation, very pronounced in the hypocotyl, gradually diminishes toward the apex of the fleshy root because there the xylem parenchyma is less abundant and the secondary growth less active. The occurrence of dilation makes it appear as though pith were present in the mature organ.

Like the xylem, the secondary phloem contains a large proportion of parenchyma (plate 11). The sieve tubes, companion cells, and phloem parenchyma occur in strands separated from each other by comparatively large parenchyma cells (plate 5). Anastomosing strands join the longitudinal bundles. The rays are just as inconspicuous in the phloem as in the xylem (plate 11). Oil ducts occur in the secondary phloem, with the secretory cells arranged in circles around the cavities.

The sieve tubes have companion cells, arranged in longitudinal series, which are shorter than the sieve-tube elements (fig. 12, *C* and *D*). The wall between a sieve tube and a companion cell is very thin, with no obvious pits (fig. 12, *B–D*). The parenchyma cells are connected by pit fields with each other and with the sieve tubes and the companion cells (figs 11, *C* and *D*, and 12, *B*). The immature sieve tubes have slime bodies, one spindle-shaped body in each element. The sieve-tube plastids contain starch that stains red with iodine. In the parenchyma cells ordinary starch occurs (plate 11).

The two strands of the primary phloem remain evident for a considerable time during the secondary growth (plates 4 and 5). The sieve tubes and the companion cells of this phloem are gradually obliterated, while the parenchyma cells enlarge and develop rather thick walls (plates 4, 5, and 10, *B*). Opposite the protoxylem poles, where no primary phloem is present, the secondary phloem is separated from the pericycle by parenchyma (plates 4 and 5). These cells arise from the cambium and, in the

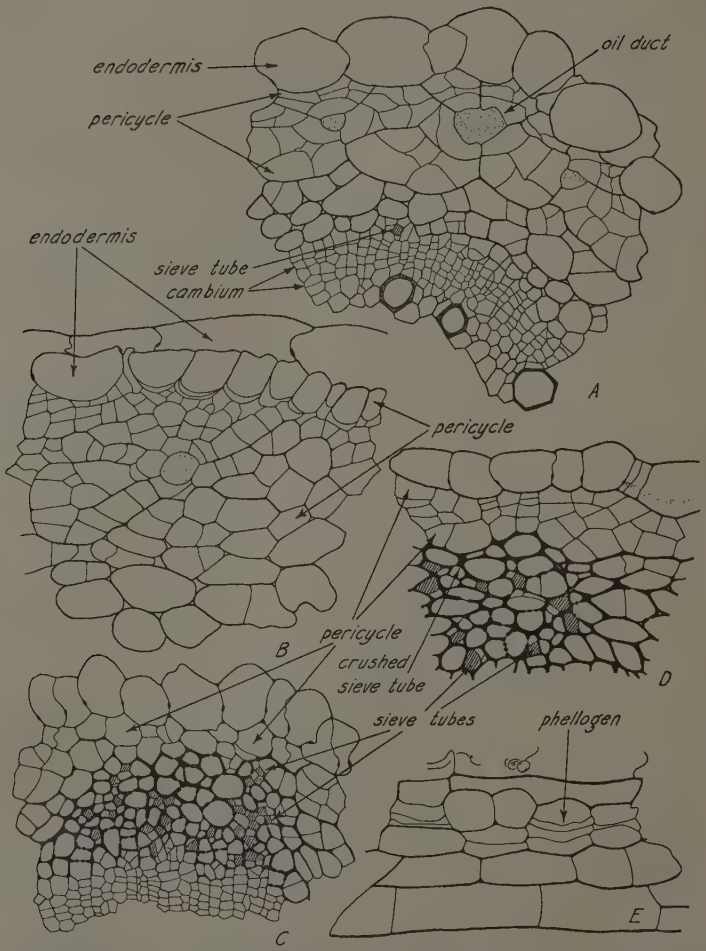

Fig. 11.—Secondary growth in the pericycle in transverse section of a hypocotyl before the rupture of the cortex. *A*, An earlier, *B*, a later stage of cell multiplication in the pericycle opposite the protoxylem. *C*, An earlier, *D*, a later stage of cell multiplication in the pericycle opposite the protophloem. *E*, Phellogen from the same hypocotyl region as in plate 14. *A* and *C* were taken from a plant similar in size to that in figure 1, *C*; *B* and *D*, as in 1, *D*; *E*, as in 2, *D*. The oil ducts are indicated by stippling; the sieve tubes by hatching. (All × 245.)

hypocotyl, also from the parenchyma originally present between the protoxylem and the pericycle.

The older secondary sieve tubes and their companion cells become functionless and are crushed. The remaining parenchyma cells elongate tangentially and divide anticlinally.

When the stele begins to enlarge through secondary growth, the cortex at first keeps pace with the increase in circumference of the organ by tangential stretching of cells and by occasional anticlinal divisions. The endoderms also shows divisions (fig. 11, *D*, and plate 4), the new walls frequently developing Casparian strips. Later the cortex, together with the endodermis, is ruptured and shed.

As was mentioned previously, the cortex sloughs first in the root, then in the hypocotyl (fig. 1, *D*, and plate 5). It persists longest on the sides along the primary phloem strands (plate 5, *A*).

When the cortex is lost, the periderm becomes the protective layer. As in most roots, this tissue layer arises in the pericycle. The secretory cells of an oil duct become subdivided into many cells, so that only a small portion of each cell remains associated with the duct (fig. 11, *A*). In shape and arrangement these new secretory cells (fig. 7, *D*) resemble the analogous cells in the secondary phloem and in the aerial parts of the plant. The first divisions of the original secretory cells occur in all planes (fig. 11, *A*) but eventually periclinal divisions predominate in the cells outside the oil ducts (fig. 11, *B*), and the groups of cells originating from one secretory cell form fan-shaped layers in cross sections (plates 5, *A*, and 10, *A*). In longitudinal sections these groups of cells appear in radial rows. Plates 9, *A*, and 12 show two stages of secondary growth in the pericycle in longitudinal sections. During the further expansion of the fleshy organ the fan-shaped arrangement is destroyed, while the cells elongate tangentially and divide radially. Toward the apex of the root, pericyclic divisions are less abundant than in the hypocotyl (plate 5).

Outside the primary phloem, where no oil ducts are present, secondary growth in the pericycle is less vigorous than elsewhere. The divisions begin here later than in the cells connected with the oil ducts (compare *A* and *C* of fig. 11) and are less numerous (compare *B* and *D* of fig. 11; *A* and *B* of plate 10). As a result of this comparatively sluggish growth, the surface of the rather young fleshy organ shows shallow grooves on the sides along the primary phloem strands (plate 5, *B*).

Periderm, defined as a tissue composed of the phellogen and its derivatives, arises in the superficial layers of the pericycle. Cells outside the oil ducts become orderly arranged because of successive periclinal divi-

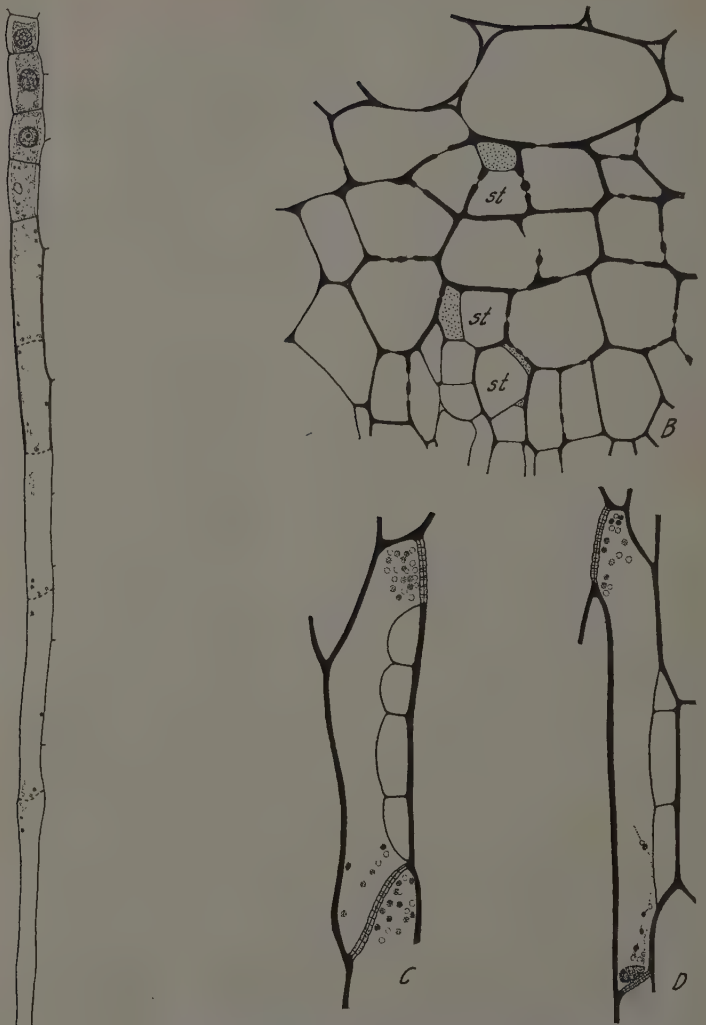

Fig. 12.—*A*, Series of protophloem sieve-tube elements. Beginning at the top: Three mother cells with nuclei; one cell with a disintegrating nucleus; two mature elements with nacré walls; and three elongated mature elements with thin walls. *B*, Secondary phloem with sieve tubes (*st*), companion cells (stippled), and phloem parenchyma from the hypocotyl of a plant like that in figure 1, *D*. *C*, *D*, Secondary sieve tubes with companion cells in tangential views. (*A* and *B*, ×605; *C* and *D*, × 472.)

sions (fig. 11, *E*, and plates 10 and 12). The outermost of these cells differentiate as cork cells, the innermost function as a cork cambium producing additional cork cells toward the periphery. Beneath the periderm are the oil ducts. These, because of the increase in circumference of the fleshy root and hypocotyl, become considerably removed from each other.

The phloem parenchyma remaining after the obliteration of the sieve tubes and companion cells, merges with the parenchyma of the pericycle lying centripetally from the oil ducts. The pericyclic cells, however, are somewhat smaller than those of the phloem (plates 12 and 13).

The pericycle, together with the periderm, forms a thin layer. The secondary xylem and the secondary phloem constitute the major portion of the storage organ (plates 13 and 14).

Since the secondary xylem and phloem contain a high proportion of parenchyma and since all their cells have rather short diameters, the tissues of the fleshy root and hypocotyl appear rather homogeneous in longitudinal and transverse sections (plates 13 and 14).

In fresh sections examined without magnification, the cambium and the youngest vascular regions together appear as a very sharply outlined uneven and narrow circle. Within the mature xylem and phloem and in the pericycle the parenchymatous areas are translucent, whereas those containing the conducting elements are opaque. The phloem is usually deeper orange than the xylem. Under the microscope the carotin crystals are seen to be most abundant in the sheets of tissue containing the sieve tubes. The phloem region also shows, to the naked eye, certain concentric opaque areas. These contain the phloem oil ducts arranged transversely and longitudinally. Their secretory cells are comparatively small and form a denser tissue than the adjacent parenchyma.

DISCUSSION

Although *Daucus carota* resembles other Umbelliferae in the peculiar structural details of the root and hypocotyl (van Tieghem, 1870–71; Warning, 1934; Hayward, 1938, chap. 15; and others), it shows fewer unusual features in the anatomy and the ontogeny of its hypogeous parts than certain other plants with fleshy storage organs. The development of the fleshy organ of the carrot occurs through the activity of a normal cambium cylinder and does not involve the occurrence of an anomalous meristematic activity as in the beet (*Beta vulgaris*) or the sweet potato (*Ipomoea Batatas*). The carrot resembles the long varieties of the radish (*Raphanus sativus*) in the morphology of the storage organ (Golinska, 1929; Hayward, 1938, chap. 10). In both, the hypocotyl and the root partake in forming this organ through excessive secondary growth; and

in both, the largest part of the hypocotyl has essentially the structure of a root. In contrast to the carrot, however, the radish, according to Hayward (1938, p. 301), has some anomalous cambial activity within the secondary xylem.

Havis (1939) interpreted the enlarged hypogeous organ of the carrot as largely hypocotyl. This region, of course, elongates considerably between the germination of the seed and the inception of secondary growth, but reaches only about 1 inch in length at the end of this period. The mature storage organ is, however, usually several inches long; hence the hypocotyl constitutes a comparatively small part of the entire structure.

The mature storage organ consists mainly of secondary vascular tissues. As Havis (1939) has indicated, the relative amount of xylem is a variable characteristic. The pericycle contributes comparatively little to the thickness of the organ. Havis (1939), on the contrary, reported a rather thick layer of pericyclic origin. It seems, he included in this layer some phloem parenchyma that remained after the obliteration of the sieve tubes and companion cells, and the parenchyma intervening between the pericycle and the first secondary phloem cells opposite the protoxylem poles.

The presence and the arrangement of the pericyclic oil ducts in the primary condition are perhaps the most unusual features of the root and hypocotyl of the carrot and other Umbelliferae (de Bary, 1884, p. 448–49; van Tieghem and Douliot, 1888; Hayward, 1938, p. 458–59; and others). Only two more families, the Araliaceae and the Pittosporaceae, are listed by de Bary (1884, p. 450–51), Courchet (1884), and Solereder (1908, p. 1101) as having a similar structure of the pericycle.

The lateral roots are initiated in the pericycle near the protophloem poles, as in figure 108, *D*, page 236 in Eames and MacDaniels' (1925) text. Courchet (1884), van Tieghem (1870–71), van Tieghem and Douliot (1888) explained the characteristic position of the lateral roots by the absence of pericyclic oil ducts opposite the phloem. As the present study shows, the first divisions in the formation of lateral roots appear in cells not connected with the oil ducts, but later the secretory cells divide and contribute to the development of the laterals and to the secondary growth in the pericycle.

Certain observations on the development of the storage organ of the carrot have a bearing upon some general questions of histogenesis and ontogeny.

The literature gives diverse data on the structure of the meristematic apices of the roots of Umbelliferae. Eriksson (1877) distinguished two

layers of initials, one giving rise to the central cylinder with its pericycle; the other to the cortex, the epidermis, and the rootcap. Holle (1876), using different kinds of roots, and van Tieghem and Douliot (1888), who studied only lateral roots, reported the presence of three sets of initials—those of the stele, those of the cortex, and the common initials of the epidermis and the rootcap. Certain recent workers similarly interpreted the meristem organization of umbelliferous roots (Warning, 1934; Hayward, 1938).

Schüepp (1926, p. 70–71), summarizing the information on root meristems, places the Umbelliferae into three different categories showing the following histogenetic relationships: (1) The cortex and the dermatogen contribute toward the rootcap (the stele, presumably, having a distinct set of initials). (2) The epidermis and the rootcap have common initials, separate from those of the cortex and those of the stele. (3) The primary tissues merge at the summit into a transverse generative layer.

Though the present study does not furnish conclusive information on the meristems of the carrot root, it indicates that the taproot of this plant, at least in seedling stage, approaches the third category listed by Schüepp. In young lateral roots, however, the stele is well marked off from the other regions. Nägeli and Leitgeb (1868) also remarked that the apical structure was clearer in lateral than in taproots, while Holle (1876) reported that the organization of the apex is sometimes more obscure in the radicle of the embryo than in the root of the seedling. The structure of root apices is obviously a complex problem. A comprehensive study of this problem should include different kinds of roots, of the same plant, in different stages of development.

In previous papers, the writer (Esau, 1938, 1939) has pointed out the close ontogenetic relationship between the pericycle and the protophloem of stems. Usually, in fact, the term *pericycle* is applied to the outer region of the phloem.

As appears from the literature on root apices, the pericycle may have independent initials or may arise from the same initials as the rest of the central cylinder; but, in any case, it is early individualized (Janczewski, 1874*a*; Flahault, 1878). In the carrot also the pericycle becomes early defined, forming a continuous layer independent of the vascular tissues until it contributes some cambium to the stele at the beginning of the secondary growth.

In some families, as in the Gramineae and Cyperaceae, the protoxylem elements lie next to the endodermis, and the pericycle appears to be a discontinuous layer. According to Janczewski (1874*a*) and Chau-

veaud (1896), however, in such families the first protoxylem elements arise from pericyclic cells, so that really the pericycle is originally continuous. If this interpretation is correct, the structure of the roots of the Gramineae and Cyperaceae, as well as the observation that the pericycle frequently has common initials with the rest of the stele, indicates a close ontogenetic relationship between the pericycle and the vascular tissues of the root.

The increase in the thickness of the root cortex of *Daucus,* through periclinal divisions of the innermost layer, and the comparatively late maturing of the endodermis are common phenomena in dicotyledonous roots (Janczewski, 1874*a;* Flahault, 1878). Monocotyledons usually show centrifugal growth in the external part of the cortex (Flahault, 1878).

The primary xylem plate of the carrot root becomes delimited before the differentiation of any vascular elements. The central vessels, commonly termed the *metaxylem,* vacuolate before the protoxylem elements, although the latter are the first to mature. Other workers have observed a similar pattern of vacuolation in many representatives of ferns, dicotyledons, and monocotyledons (Russow, 1872, p. 17; Janczewski, 1874*a*; Chauveaud, 1896, 1900; Stover, 1928; Esau, 1935; Hayward, 1938, p. 295). Nevertheless the primary xylem of roots is interpreted as having a centripetal order of differentiation, because such is the order of development of the secondary walls and of the loss of protoplasts—that is, because the peripheral elements become specialized and begin to function before the inner ones.

The first protophloem sieve tubes of the carrot root mature earlier than the first protoxylem elements; in other words, they lie closer to the terminal meristem than do the water-conducting elements. This observation agrees with the conclusions of several other workers who have studied vascular differentiation in root tips. (See review by Esau, 1939.)

Since the first two sieve tubes resemble the pericyclic oil ducts in their shape and clearness of contents, they have often been interpreted, in the Umbelliferae, as secretory canals (Courchet, 1884; van Tieghem and Douliot, 1888; Warning, 1934; Hayward, 1938, p. 459, 461). Crooks (1933) also regarded as ducts the first phloem elements in the flax root. In the carrot, however, as the present study shows, the cells in question are sieve tubes with all the usual characteristics of these elements.

The position and the characteristic appearance of the first protophloem sieve tubes of roots have been studied in much detail by Chauveaud (1896, 1900, 1903) in numerous representatives of cryptogams, gymnosperms, and angiosperms. These contributions have markedly fa-

cilitated the recognition and interpretation of the first phloem elements in differentiating roots.

In the development of the lateral root the behavior of the endodermis of the main root deserves some attention. In the carrot this tissue layer forms a temporary covering of the young root and is discarded as the organ emerges on the surface of the main root. A similar development of the endodermis has been observed in other Umbelliferae by van Tieghem and Douliot (1886*b*, 1888), who interpreted the layer derived from the endodermis as a digestive pocket.

The endodermis frequently participates in the formation of lateral roots in dicotyledons and monocotyledons (Nägeli and Leitgeb, 1868; Lemaire, 1886; van Tieghem and Douliot, 1886*a*, 1886*b*, 1888; Crooks, 1933; Hayward, 1938, p. 51–52). According to Janczewski (1874*b*), plants vary considerably in the degree of this participation; but usually the endodermis forms a single exterior layer.

As van Tieghem and Douliot (1888) have observed in other plants, the lateral root of the carrot forms its own endodermis in connection with the endodermis of the main root.

A study of differentiation of the vascular tissues involves the problem of distinguishing between the different kinds of primary and between the primary and the secondary tissues. Russow (1872, p. 3) introduced the name *protoxylem* for the first mature xylem elements, which, in the root, appear farthest away from the center of the axis. The term *metaxylem* was apparently first used by van Tieghem (1887) who applied it to the centrifugal xylem elements that occupy the same position as the secondary elements but are not yet derived from the cambium. Plants may or may not have such metaxylem. Van Tieghem regarded the entire centripetal xylem plate as protoxylem, apparently without considering the nature of the secondary walls.

In current usage, the term *metaxylem* is applied, in roots, to the innermost centripetal vessels (Jackson, 1928); and only the outermost groups represent the protoxylem. The secondary xylem is, of course, the tissue derived from the vascular cambium. Definite wall sculpturings are ascribed to the different kinds of xylem (Eames and MacDaniels, 1925, p. 92).

In the carrot the innermost vessels are scalariform and reticulate, the latter type being—according to the current concept—a representative of the metaxylem. The cambium, however, produces largely scalariform and some reticulate vessels—that is, elements considered characteristic of the primary xylem. Apparently, therefore, a certain kind of wall sculpturing is not necessarily correlated with the method of formation

of xylem elements, and the distinction between the different kinds of xylem is uncertain. A similar difficulty exists in the attempts to distinguish between the protophloem, the metaphloem, and the secondary phloem.

As the writer (Esau, 1938) has previously emphasized, the distinction between the cambium and procambium in stems and leaves is uncertain because the vascular tissues called *primary* commonly arise from a radially seriated meristem. In the root the entire central cylinder is interpreted as procambium. This meristematic region seems to fit the definition of procambium better than does the corresponding vascular meristem in the stems and leaves, since in the former the walls are formed in many planes and the divisions are largely completed before the appearance of any mature vascular elements.

In this respect it seems significant that, as exemplified by *Daucus,* the centripetal xylem and the corresponding phloem—tissues not derived from a radially seriated meristem—are connected only with the cotyledons. The plumule, developing after the primary body of the seedling plant has been practically completed, forms vascular connections with the secondary tissues of the hypocotyl and root (perhaps also with the small fraction of primary xylem differentiating centrifugally). Thus the collateral arrangement and the radial seriation of the vascular tissues are continuous from the root into the aerial parts of the plant above the cotyledons. Such a relation is, of course, not only true of *Daucus* but probably common in the dicotyledons (Chauveaud, 1911; Havis, 1935, 1937; McMurry and Fisk, 1936; Simonds, 1938).

Apparently, then, homologous vascular tissues are interpreted as primary in the leaf and stem and as secondary in the root. In criticizing this concept, Chauveaud (1911) suggested that the vascular tissues of stem and leaf should be regarded as largely secondary. Studies of seedling anatomy considering the ontogeny and interconnection of xylem and phloem in different parts of the plant would be particularly valuable for the solution of the problem of distinguishing between the primary and the secondary vascular tissues.

SUMMARY

In the primary state the root of the *Daucus carota* L. shows a diarch xylem plate and two strands of phloem lying opposite each other. Vascular differentiation begins with the delimitation of the xylem plate through expansion and vacuolation of the future vessels. Though the central vessels vacuolate first, they are last to develop secondary thickenings and to lose their protoplasts. After having been set aside, the xylem

mother cells do not divide longitudinally any further; but the cells on both sides of the plate—that is, in the two future phloem regions—divide in all planes until the maturation of the vascular elements sets in.

The first mature elements are two protophloem sieve tubes lying opposite each other next to the pericycle in a plane at an angle of 90° to the future xylem plate. In seedlings grown in moist chambers in the laboratory the sieve tubes complete their differentiation about 300 microns from the apex of the root. The first protoxylem vessels, also located next to the pericycle, show secondary walls approximately 1 millimeter from the root apex, but do not lose their protoplasts through another millimeter of the root.

A centripetal differentiation of additional xylem and phloem elements follows, and a few centrifugal xylem elements complete the primary vascular development of the root.

The pericycle becomes individualized before the beginning of vascular differentiation and, through a series of oblique divisions, gives rise to oil ducts. These are arranged in two arcs, each with its central oil duct opposite a protoxylem pole.

The cortex, about two cells wide near the apex, increases in width to four or five cells by tangential divisions of the innermost cortical layer. Thus the endodermis is the last cortical layer formed and develops Casparian strips in the region where the first vessels reach maturity.

The outermost cortical layer divides periclinally near the apex of the root, the outer daughter cells becoming part of the rootcap, the inner differentiating into epidermal cells.

Throughout most of its extent, the hypocotyl resembles the root. The change from the centripetal to the centrifugal order of xylem differentiation occurs in the cotyledons, and the primary vascular tissues branch into the cotyledonary traces just below the insertion of the cotyledons. The xylem, however, shows an increasing parenchymatization from the base of the root toward the top of the hypocotyl; the phloem strands become wider; and the entire stele increases in diameter. In the upper hypocotyl the Casparian strips disappear, and starch accumulates in the endodermis.

The centripetal xylem and the corresponding phloem are connected with the cotyledons only. The plumule forms direct vascular connections with the secondary vascular tissues of the root and also, possibly, with the centrifugal primary xylem.

The lateral roots of the taproot and the hypocotyl are initiated near the protophloem poles in the part of the pericycle that lacks oil ducts, but later the nearest secretory cells also contribute to the growth of these

roots. The endodermis partakes in the lateral root development by forming a temporary covering over the young organ. It is destroyed as the root emerges on the surface. At the same time the lateral root develops its own endodermis and also its primary vascular tissues. The endodermis and the xylem and phloem of the lateral root are connected with the corresponding tissues of the main root. Some lateral roots arise in the periderm after the secondary growth has occurred in the root and the hypocotyl.

The fleshy underground storage organ of the carrot is formed through secondary growth in the taproot and the hypocotyl. The cambium, arising in the usual manner between the primary xylem and primary phloem, forms highly parenchymatous secondary vascular tissues. During this growth the cortex is ruptured and shed. The pericycle, through active divisions externally and internally to the oil ducts, gives rise to several layers of parenchyma. Outside the oil ducts the parenchyma cells give rise to a phellogen producing cork after the cortex sloughs. As the tissues arising from the pericycle form together only a narrow zone, the fleshy storage organ consists mainly of the secondary vascular tissues.

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PLATES

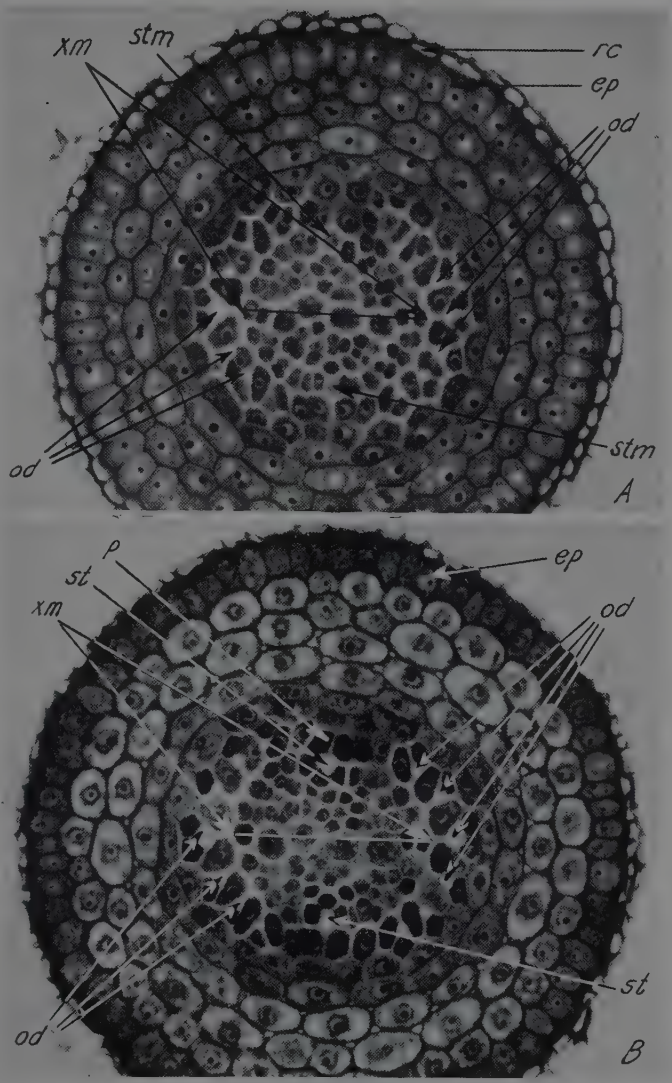

Plate 1.—Transverse sections of a root apex of a seedling, showing structure before (*A*) and after (*B*) differentiation of the first two protophloem sieve tubes. The section in *B* was taken 300 microns from the apex of the root. Details are: *ep*, epidermis; *od*, oil duct; *p*, pericycle; *rc*, rootcap; *st*, sieve tube; *stm*, sieve-tube mother cell; *xm*, xylem mother cells. (Both × 400.)

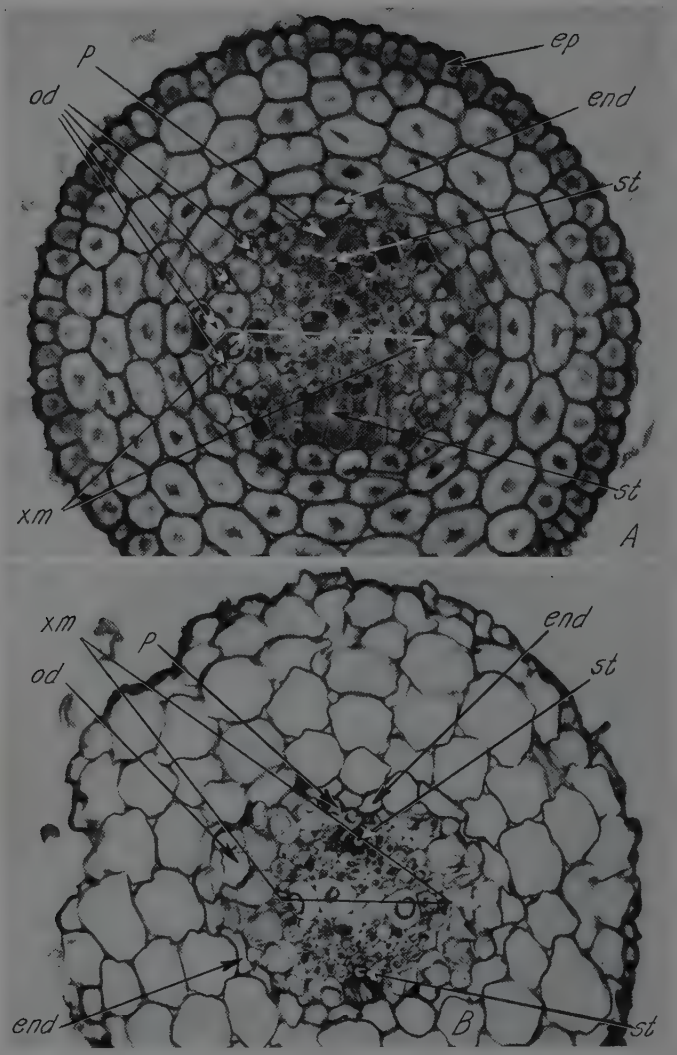

Plate 2.—Transverse sections of a root taken at successively higher levels of the same seedling as in plate 1. Details are: *end*, endodermis; *ep*, epidermis; *od*, oil duct; *p*, pericycle; *st*, sieve tube; *xm*, xylem mother cells. (Both × 400.)

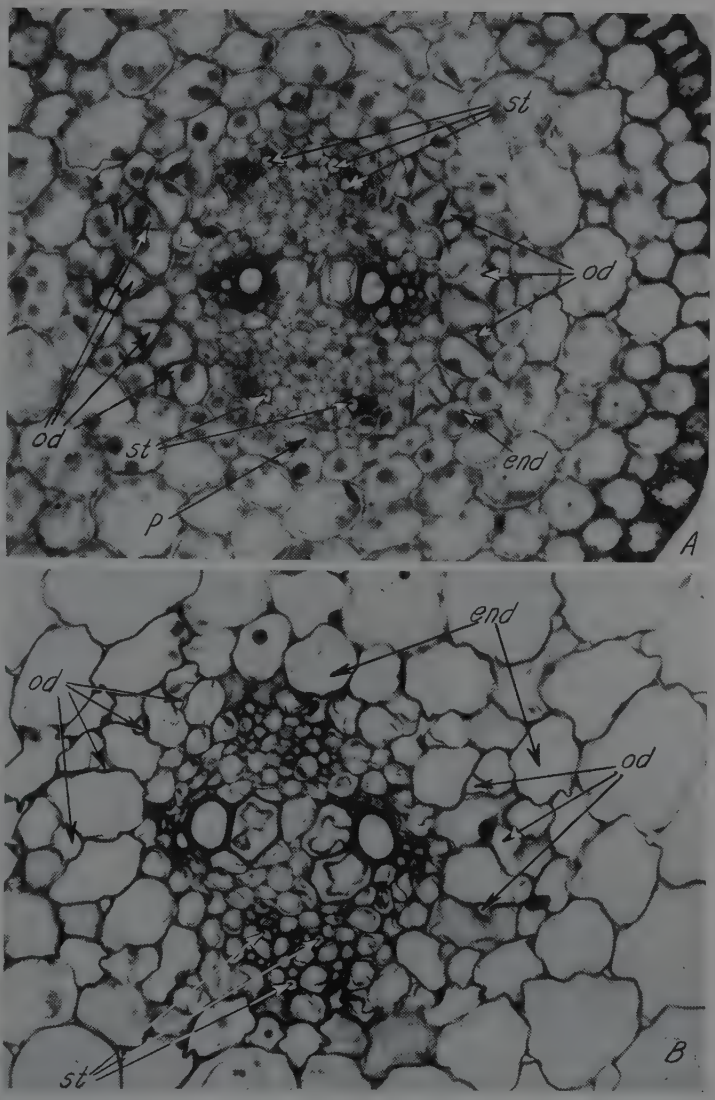

Plate 3.—Transverse sections of the hypocotyl of the same seedling as in figure 6. *B* was taken near the root base; *A*, farther away. The dark bodies in the endodermis and the cortex of *A* are plastids with starch grains. *A* shows a few epidermal cells to the right. Details are: *end*, endodermis; *od*, oil duct; *p*, pericycle; *st*, sieve tube. (Both × 400.)

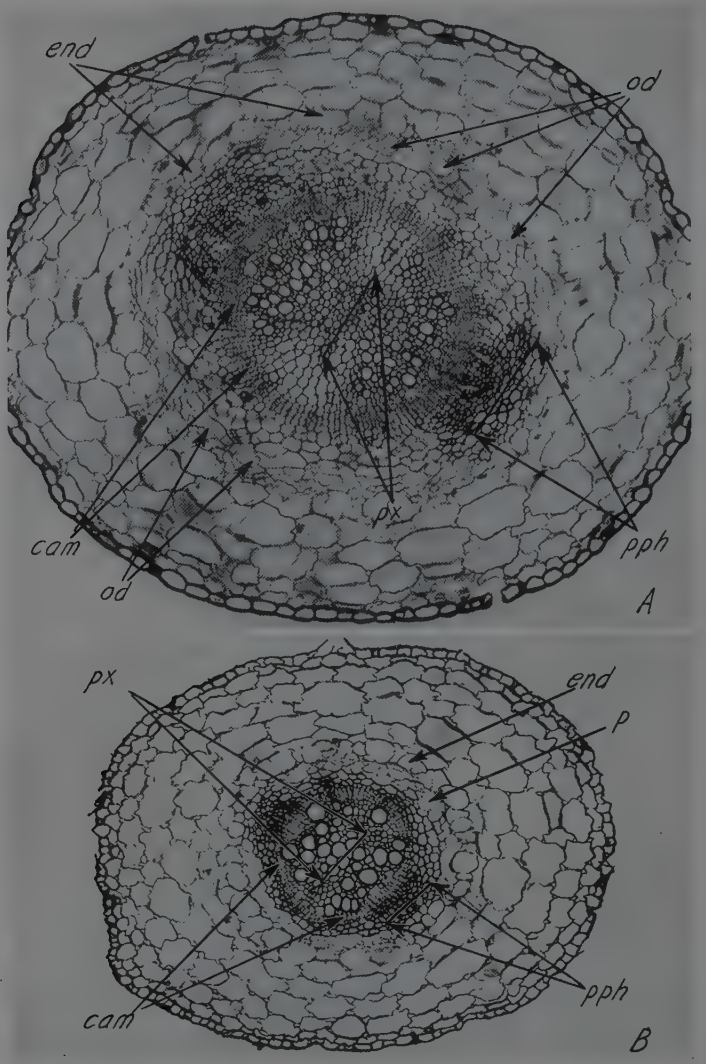

Plate 4.—Transverse sections showing the structure of hypocotyl (*A*) and root (*B*) of a plant like that in figure 1, *C*. Some secondary vascular tissues have been produced, but the cortex is still intact. Details are: *cam*, cambium; *end*, endodermis; *od*, oil duct; *p*, pericycle; *pph*, primary phloem; *px*, primary xylem. (Both × 92.)

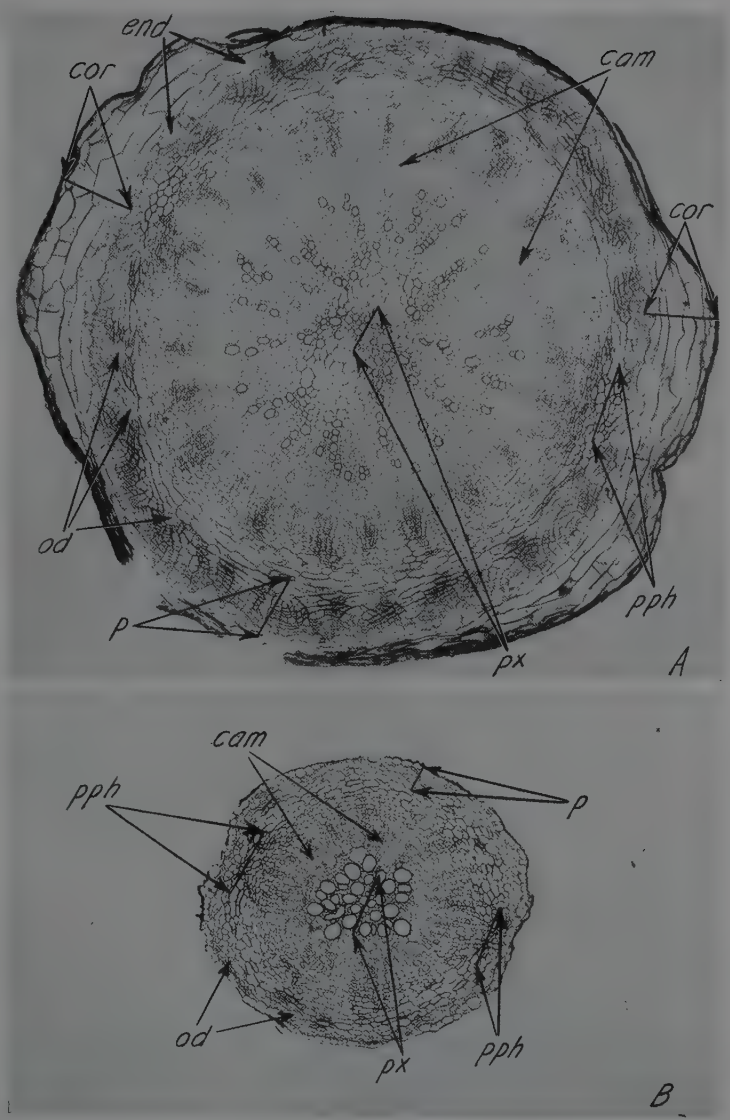

Plate 5.—Transverse sections of hypocotyl (*A*) and root (*B*) of a plant like that in figure 1, *E*. In the hypocotyl some of the cortex is still present; in the root this tissue region has sloughed. Details are: *cam*, cambium; *cor*, cortex; *end*, endodermis; *od*, oil duct; *p*, pericycle; *pph*, primary phloem; *px*, primary xylem. (Both × 50.)

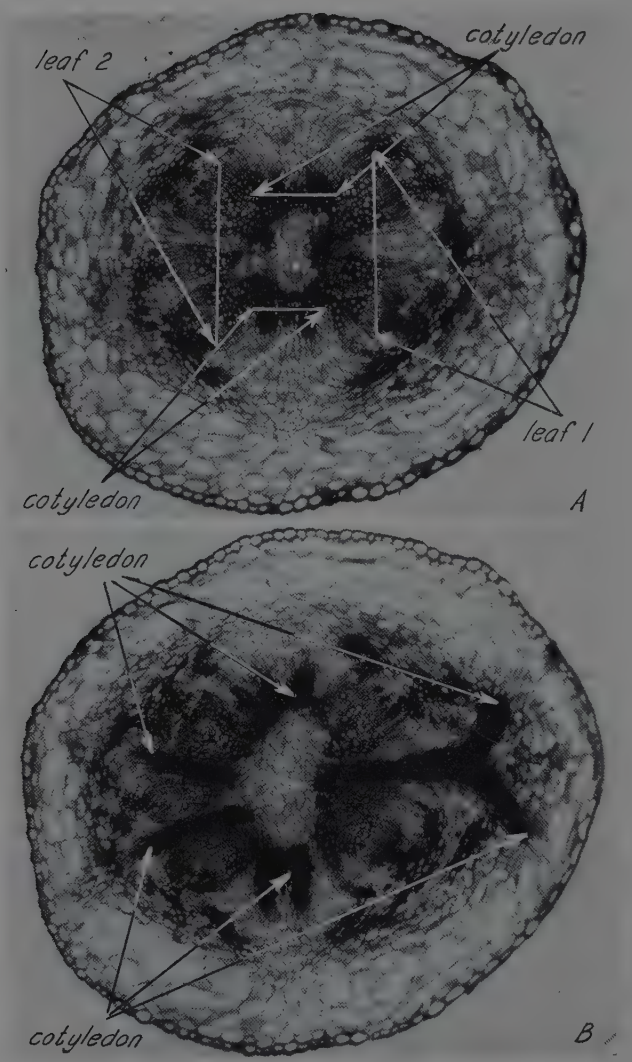

Plate 6.—Transverse sections of upper hypocotyl of a plant like that in figure 1, *C*. *A* was taken at a lower, *B* at a higher level below the cotyledons of the same plant as in plate 4. The sections show the arrangement of traces of the cotyledons and of the first two foliage leaves. Compare with figure 7, *C* and *D*. (Both × 50.)

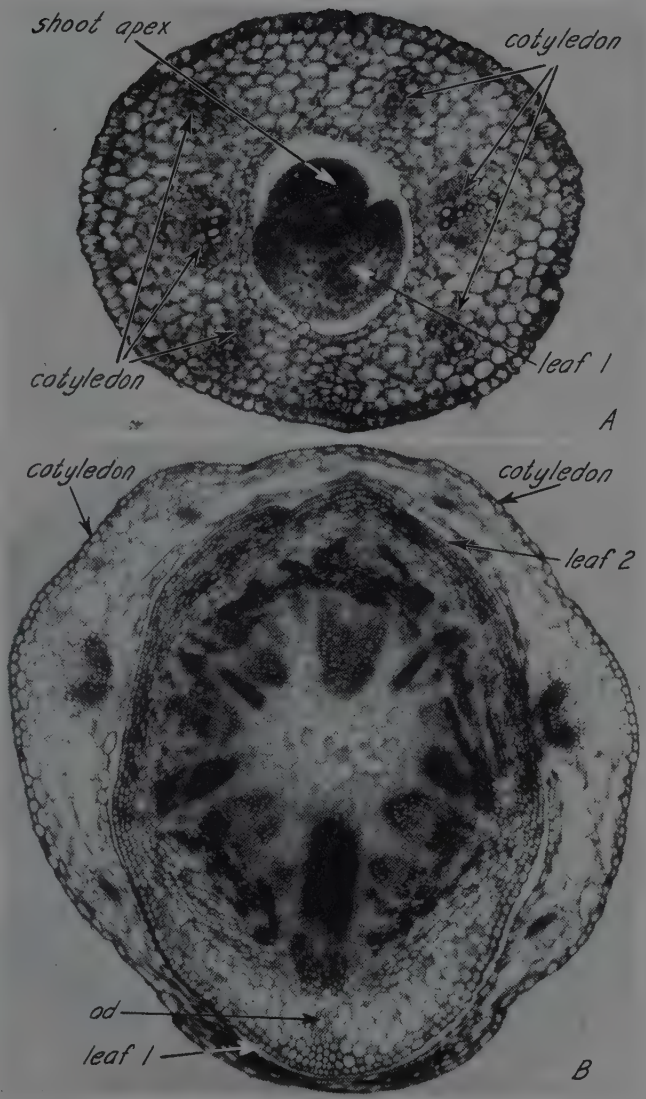

Plate 7.—Transverse sections of a younger (*A*) and an older (*B*) plant showing the structure of the axis above the insertion of the cotyledons. *B* was taken from the same plant as the sections in plates 4 and 6. The bases of the cotyledonary petioles are fused. (*A*, × 168; *B*, × 50.)

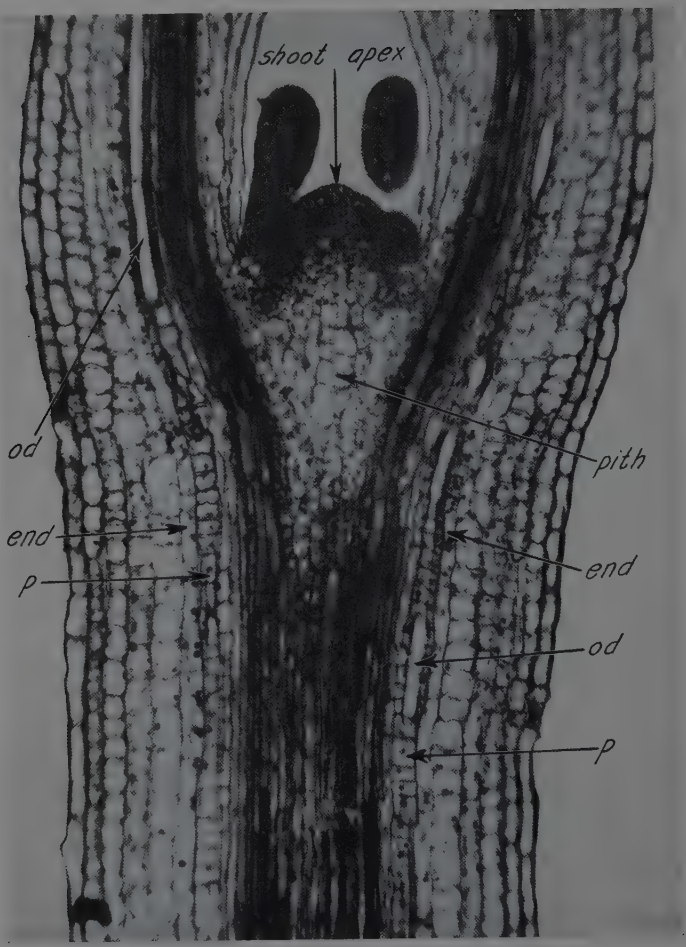

Plate 8.—Longitudinal section of the epicotyl and upper hypocotyl of a plant similar in size to that used for plate 3. This section shows the union of the cotyledonary traces below the primordial shoot. Details are: *end*, endodermis; *od*, oil duct; *p*, pericycle. (× 188.)

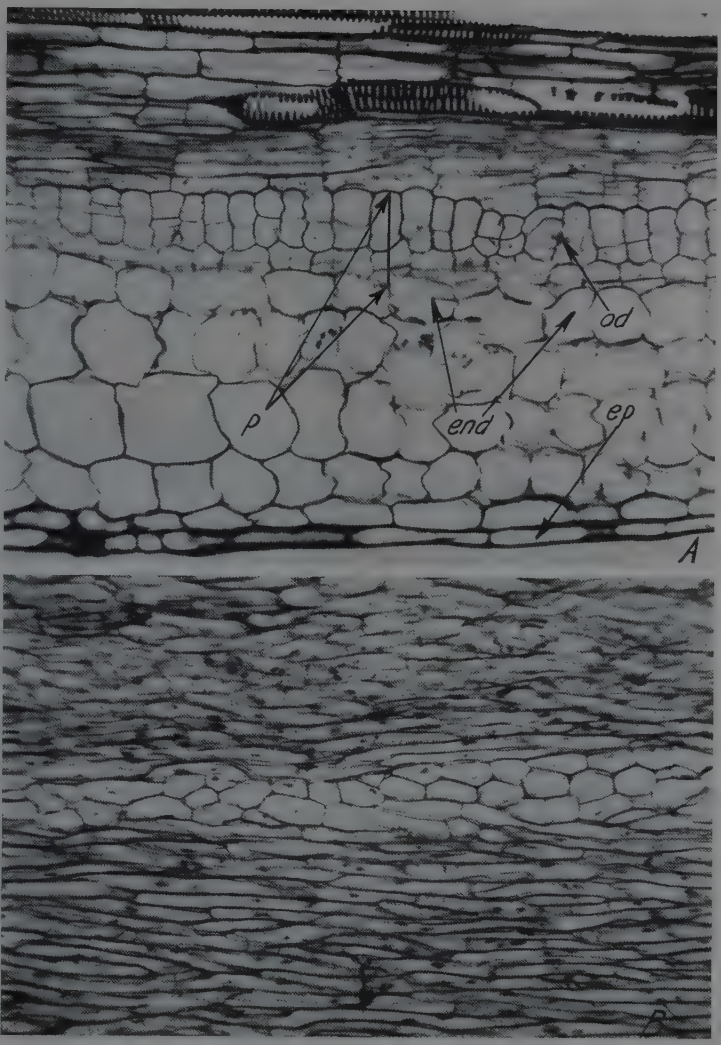

Plate 9.—*A*, Longitudinal section of the lower hypocotyl of a plant like that in figure 1, *C*. A similar region appears in transverse section in plate 4, *A*. Some secondary cell division has occurred in the pericycle. The longitudinal section does not include the primary phloem but shows some cambium below the xylem. *B*, Tangential section of the vascular cambium from the root of a plant like that in figure 2, *A*. The large, nearly isodiametric ray initials contrast sharply with the fusiform initials. Details are: *end*, endodermis; *ep*, epidermis; *od*, oil duct; *p*, pericycle. (Both × 188.)

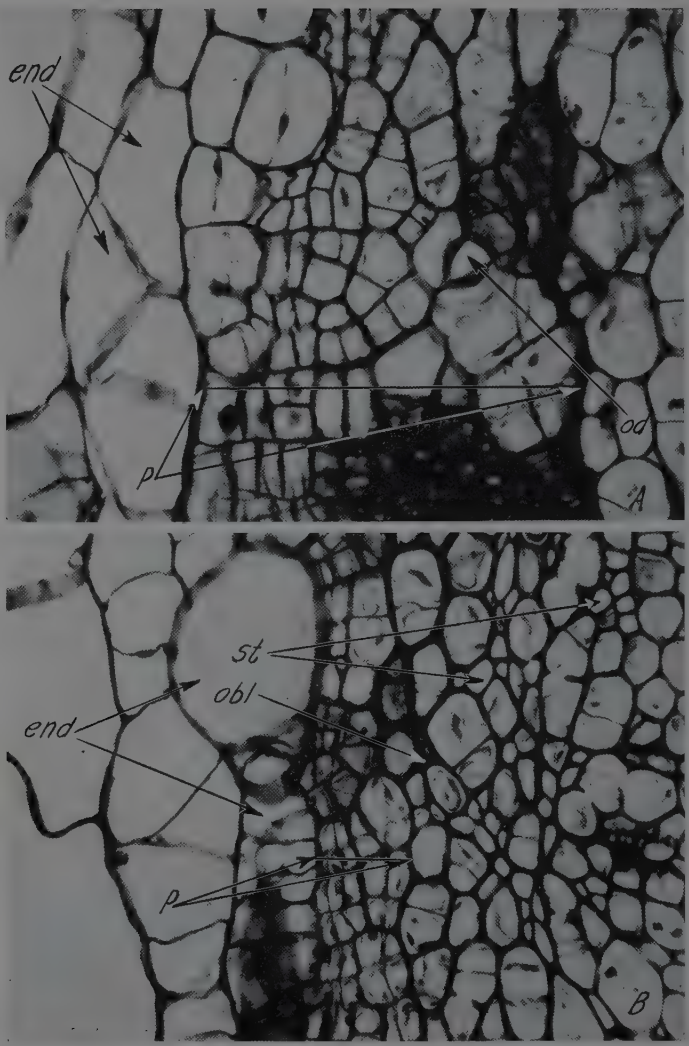

Plate 10.—Transverse sections of a hypocotyl showing cell multiplication in the pericycle before rupture of the cortex. Earlier stages of this process are shown in figure 11. *A* was taken opposite the primary xylem; *B* opposite the primary phloem, from a plant like that depicted in figure 1, *D*. Details are: *end*, endodermis; *obl*, obliterated elements; *od*, oil duct; *p*, pericycle; *st*, sieve tube. (Both × 400.)

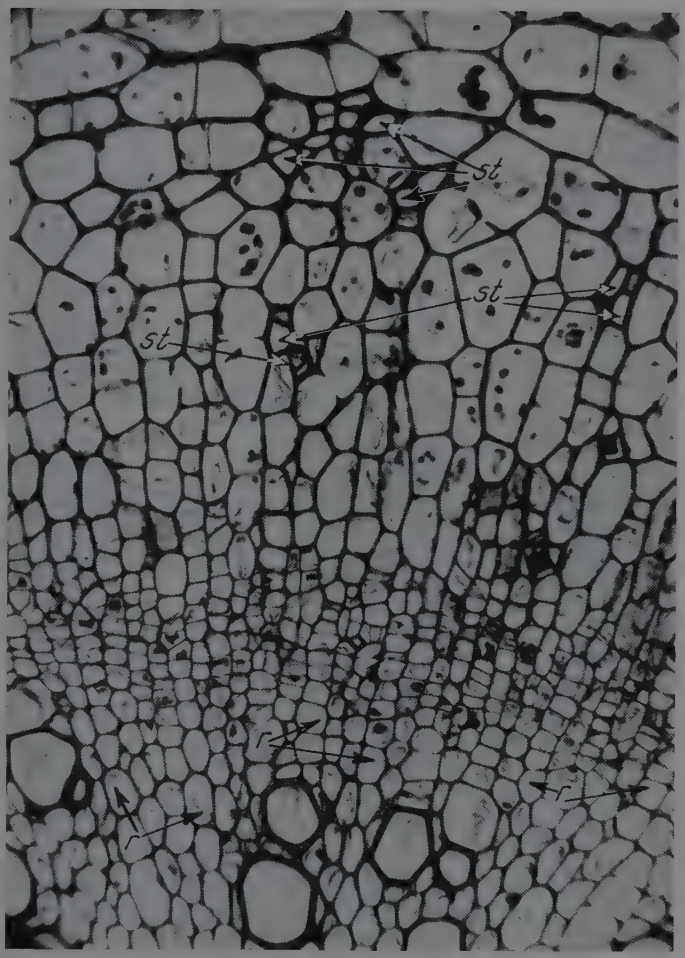

Plate 11.—Transverse section of secondary vascular tissues of the hypocotyl of a plant like that in figure 1, *D*. This photograph was taken from the same section as those in plate 10. Details are: *r*, ray; *st*, sieve tube. (× 400.)

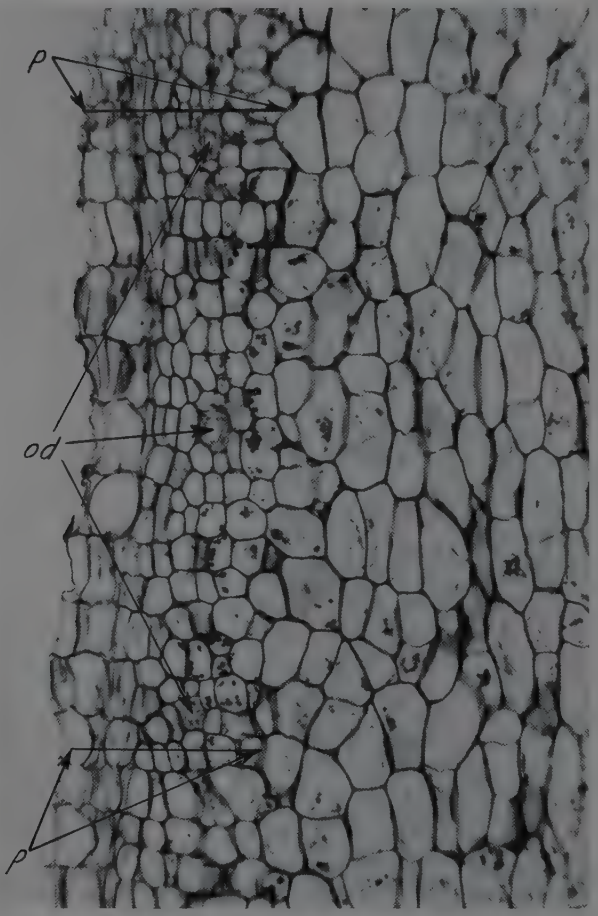

Plate 12.—Radial longitudinal section of outer phloem and pericycle with periderm of the root of a plant like that in figure 2, *A*. Details are: *od*, oil duct; *p*, pericycle with periderm. (× 188.)

Plate 13.—Radial longitudinal section of root of a plant somewhat smaller than that in figure 2, *C*. Details are: *cam*, cambium; *p*, pericycle with periderm; *ph*, phloem; *r*, ray; *x*, xylem. (× 16.)

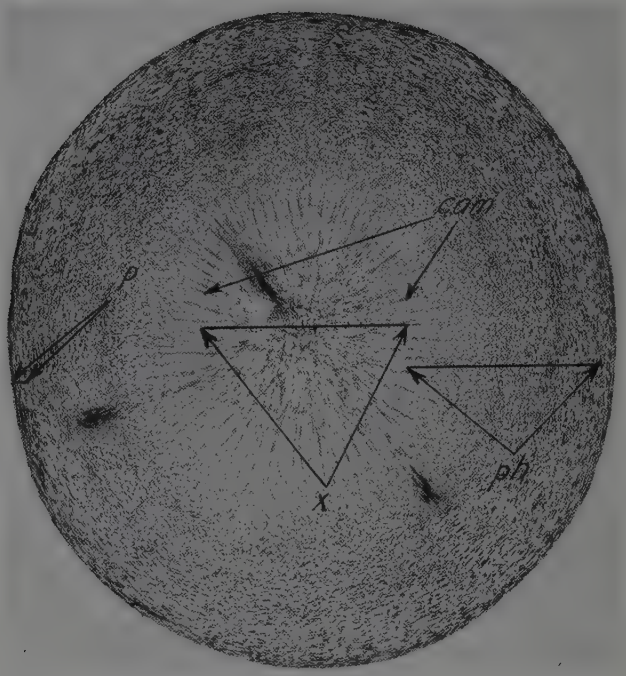

Plate 14.—Transverse section of hypocotyl 1 centimeter below the insertion of leaves in a plant like that in figure 2, *D*. Details are: *cam*, cambium; *p*, pericycle with periderm; *ph*, phloem; *x*, xylem. (× 6.)

STRUCTURE OF END WALLS IN DIFFERENTIATING VESSELS

KATHERINE ESAU AND WM. B. HEWITT

STRUCTURE OF END WALLS IN DIFFERENTIATING VESSELS[1]

KATHERINE ESAU[2] AND WM. B. HEWITT[3]

INTRODUCTION

THE SENIOR WRITER'S observations on the method of establishment of continuity between vessel segments in celery (Esau, 1936*b*)[4] contradicted Priestley and his associates' (1935; 1938, p. 348) interpretation of this phenomenon in other plants. It seemed desirable, therefore, to survey the literature for references on vessel ontogeny and to check the results obtained with celery by using some other plants. Moreover, addition of microchemical tests to the morphologic observations appeared pertinent. These enlarged studies on the nature and longevity of vessel end walls are described in the present paper.

REVIEW OF LITERATURE

The development of continuous xylem tubes by perforation or complete removal of end walls in series of superposed cells has been recorded by early workers (Treviranus, 1835; Mirbel, 1837; and others). Among these, von Mohl (1845*a*, 1845*b*, 1849) and Crüger (1855) observed that the continuity between the vessel elements was established after the development of the secondary thickenings[5] on the longitudinal walls and after the formation of the rim of secondary nature on the end walls. Many of the subsequent workers also observed that the end walls were perforated during the final stages of vessel differentiation, that is, after the vessel elements attained their mature diameter and maximum wall thickness (de Bary, 1884, p. 165; Strasburger, 1882, p. 81; Lange, 1891; Flach, 1924; Eames and MacDaniels, 1925, p. 151; Duerden, 1934; Esau, 1936*b*).

Hartig (1853) and Flach (1924) reported that the dissolution of the

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[4] See "Literature Cited" for complete data on citations, which are referred to in the text by author and date of publication.

[5] The terms *secondary thickening* or *secondary wall* are used throughout this paper to denote the rings, spirals, reticulae, or the more extensive pitted layers of wall material, that are laid over the continuous (except for the plasmodesmata perforations), commonly thin wall of the expanded vessel mother cell. The continuous wall is referred to as the primary wall.

end walls was a gradual process beginning in one spot—in the middle of the wall, according to Flach—and spreading thence to other parts of the wall. The protoplasts of the individual vessel elements disintegrated approximately at the same time as the end walls (Hartig, 1853; Flach, 1924; Eames and MacDaniels, 1925, p. 151, fig. 75; Esau, 1936*b*). Workers noted that walls undergoing disintegration were rarely encountered in preparations (Flach, 1924; Duerden, 1934; Esau, 1936*b*).

Long before the end wall breaks down it becomes much thicker than the primary longitudinal walls (Strasburger, 1882, p. 81; Flach, 1924; Esau, 1936*b*). If only a portion of the end wall is removed the thickening is restricted to this portion (Esau, 1936*b*). Strasburger (1882, p. 81) and Flach (1924) interpreted this thickening as swelling. According to Flach, during this swelling the hemicelluloses of the end wall are converted into pectic materials. Pure cellulose was said to be absent in the end walls.

Eames and MacDaniels (1925, p. 151, fig. 75*c*) depicted the end-wall thickening in differentiating vessels as secondary; Flach (1924) and Esau (1936*b*) regarded it as primary.

In contrast to the writers considered above, Priestley and his coworkers (1935, 1938, p. 348) advance the concept that end walls in vessels of woody angiosperms are destroyed during the expansion of the mother cells. These authors have not seen fragments of such walls, but suggest that the walls break in the middle and then rapidly retract toward the periphery, and thus form a thick rim around the orifice. Their figures 2 and 3 (Priestley, Scott, and Malins, 1935) supposedly show such rims. In figure 3, however, the "rim" resembles the thickened lenticular end wall described by Esau (1936*b*, plates 2, *A*, and 4, *A–C*). In the same figure one might suspect the presence of two fragments of the end wall hanging down into the lower cell, but these structures are also labeled as rim.

Although, according to Priestley, Scott, and Malins (1935, p. 45, 53), the end wall is perforated early in vessel development, the protoplasts of the superposed segments do not mingle, for "thin, mucilaginous pectin films" often remain stretched across the opening. The structures labeled as films in plate 1, figures 2 and 3, of these authors, however, appear like end walls cut on slant or torn in cutting.

References to breakdown of end walls occur also in the group of papers describing a multinucleate phase in vessel development of some plants.

According to Kny (1886), in certain Dioscoreaceae and woody Liliaceae the very long tracheids of the secondary bundles of stems are formed from series of superposed cells in which transverse walls disintegrate.

The phenomenon is said to occur before secondary walls are laid down and while the protoplasts are alive. A multinucleate condition results from fusion of protoplasts.

Although certain later workers (Wieler, 1889; and others) accepted Kny's interpretation, others did not. Among the latter Röseler (1889), having reinvestigated the Liliaceae used by Kny, concluded that the tracheids attain their length through gliding growth and that the multinucleate condition is only apparent; the nuclei belong to different radially superposed cells. Scott and Brebner (1893) and recently Cheadle (1937) agreed with Röseler that the tracheids arose from single uninucleate cells elongating by sliding growth.

Hill and Freeman (1903) described a multinucleate condition in young vessels of *Dioscorea prehensilis* and said it was produced by the obliteration of walls between the superposed cells. Pirotta and Buscalioni (1898), however, reported that the vessel elements of Dioscoreaceae became multinucleate through repeated nuclear divisions and that the perforation of the end walls occurred after the nuclei disintegrated. Němec (1910) agreed with this interpretation.

In the first of two articles on vessels of *Ricinus,* Scott (1935) reported that the young vessels became coenocytic through an early breakdown of transverse walls. The second paper (Scott, 1937, p. 71) implies that omission of cytokinesis in procambium cells causes the multinucleate condition.

The multinucleate vessel mother cells of Euphorbiaceae, including *Ricinus,* were previously considered by Smolák (1904). According to this worker, nuclei multiply without formation of cell walls and the vessel end walls are resorbed after the secondary layers appear on the longitudinal walls. Smolák's observations were confirmed by Němec (1910, p. 120–151).

In the writers' opinion, workers who place the establishment of continuity in a vessel at the end of its differentiation have furnished more convincing data to support their contention than those who suggest that the perforation of the end wall occurs while the vessel mother cell is expanding. The observations here recorded further show the persistence of terminal walls in differentiating vessels.

MORPHOLOGY OF THE END WALLS IN DIFFERENTIATING VESSELS

The characteristics and behavior of vessel end walls were studied in several herbaceous angiosperms—*Cucurbita pepo, Zea mays, Nicotiana tabacum, Daucus carota,* and *Beta vulgaris.*

Cucurbita belongs to the type of plants in which the differentiating vessels expand very rapidly, attaining much larger diameters than the meristematic cells from which they arose. Plate 1, *A*, illustrates the striking contrast in size between the cambium cells and the vessels.

The enlarging vessels, of course, affect the arrangement of adjacent cells; the latter are stretched transversely and are also pulled apart. The separating cells remain, however, partly attached to each other by means of cell prolongations that appear like protuberances or arms (plate 3, *C*). Zimmermann (1922) has amply illustrated the bizarre shapes encountered among these cells.

Through the separation of adjacent cells the vessel is brought into contact with new cells (Velten, 1875; Priestley, Scott, and Malins, 1935; Esau, 1936*a*; and others).

Regardless of the rapid and immense expansion of the large vessels of *Cucurbita*, the end walls do not break down during this growth. On the contrary, after the completion of vessel enlargement their transverse walls are thicker and therefore more prominent than the longitudinal walls (plate 2, *B* and *C*). At this time the end walls appear tightly stretched; the longitudinal walls are pulled in at the transverse partitions so that the vessel elements appear constricted at the ends.

Plate 2, *C*, shows that the end walls are much thinner at their margins than in the median portion. These margins remain intact in mature vessels and are covered by rims of secondary wall material. In the relatively narrow celery vessels studied previously (Esau, 1936*b*) the end walls were thickest in the middle and tapered gradually toward the margins so that the walls resembled biconvex lenses. This shape of the end walls is apparently characteristic of vessels of narrow diameter and was observed in the primary xylem of *Beta*, *Daucus*, and *Nicotiana* used in this study.

The end walls persist until secondary layers develop on the longitudinal walls. The *Cucurbita* vessel mother cells shown in plate 2, *A*, had secondary walls with bordered pits; but their transverse walls and protoplasts were still intact. In this photograph seven transverse end walls appear in section; the other five are somewhat tilted, appearing like thin films.

In *Zea mays* the expansion of the two large pitted metaxylem vessels is also rather marked. In the vascular bundle from a stem of maize in plate 1, *B*, these vessels had no secondary walls but were fully expanded. One of the two large vessels showed the transverse end wall covered with cytoplasm.

Plate 4, *A* and *B*, illustrates portions of metaxylem vessels of maize in

longitudinal sections. The element in *A* had secondary thickenings on longitudinal walls, but was cut mostly through parts of walls that bore no pits. The primary end wall in this view is as thick as the secondary longitudinal walls. The rims seen in section right and left on the transverse wall are secondary and are rather sharply set off from the primary end wall.

Traces of deeply stained material appear in place of the terminal wall in plate 4, *B*. In contrast to the cells in *A*, those in *B* are free of cytoplasm.

In the vessel cells in *B*, bordered pits are perceptible in the longitudinal walls and occur also in the rather wide rim.

Plate 3, *B*, shows longitudinally a portion of a young pitted vessel from the secondary xylem of *Nicotiana*. This vessel had reached its ultimate width and had secondary walls. The part of the oblique end wall that would have been removed upon maturation of the vessel is black in plate 3, *B*. Because it was cut somewhat on slant, its thickness is exaggerated.

The section in plate 3, *B*, was stained with safranin and anilin blue and then photographed through Wratten filters 25A and 52 naphtol green. In the photograph the lignified secondary walls, which were stained pink, came out faintly gray. The pit-closing membranes and the primary portion of the end wall were stained blue and appear black.

Only elements of the primary xylem were examined in the sugar beet and carrot. These two plants gave views very similar to those obtained with the celery (Esau, 1936*b*).

MICROCHEMICAL AND OPTICAL TESTS OF THE END WALLS

The primary purpose of the microchemical and optical tests was to determine whether or not cellulose was present in those vessel walls that disintegrate when the vessels mature. These studies were made by the methods described by Rawlins (1933). Before the tests, the material was imbedded in paraffin, sectioned with a microtome, 8 to 10 microns thick, mounted on slides, and treated with xylene and alcohol. The vessel end walls of all the three plants used in these studies—*Cucurbita*, *Zea*, and *Nicotiana*—showed similar reactions.

Cellulose is doubly refractive, or anisotropic when observed between crossed Nicol prisms. A transverse or oblique partition between two vessel elements consists of two peripheral anisotropic layers and a median isotropic (not doubly refractive) layer. The anisotropic layers are present before and after expansion of the vessel element. A treatment of the

sections for 15 to 20 minutes in water just below the boiling point enhanced the prominence of the doubly refractive layers (plate 4, *C*). They became particularly bright after the sections were stained with congo red (plate 4, *D*) or with iodine in potassium iodide[6] and duPont Rayon Bordeaux B.

Either zinc-chlor-iodide or I_2KI followed by 65 per cent H_2SO_4 stains the cellulose blue; the lignified and the suberized walls yellow. Both reagents stain the anisotropic layers blue, the zinc-chlor-iodide giving a more intense coloration than the I_2KI. Plate 3, *A*, shows a portion of *Zea* vessel treated with zinc-chlor-iodide. The blue layers appear as thin dark lines on either side of the thick transverse partition.

Cellulose is soluble in either cuprammonia solution or in 72 per cent H_2SO_4. These reagents removed the anisotropic layers of the vessel end walls but left the isotropic substance.

Sections exposed to alternate treatments with chlorine water and hot 2 per cent sodium sulfite—a treatment that dissolves lignin and the intercellular material but leaves the cellulose—left the two anisotropic layers of the vessel end walls intact. The isotropic layer was apparently removed; it could not be detected by staining with ruthenium red, hematoxylin, or congo red after the treatment.

Under certain conditions hemicellulose shows anisotropy. It is, however, soluble in dilute mineral acids and alkalies. After treatment with either 3 per cent KOH or 6 per cent HCl at room temperature the end walls of the vessel elements swelled but neither the anisotropic nor the isotropic layer was dissolved.

The entire end wall of a vessel element stains red with ruthenium red. This staining reaction probably indicates the presence of the so-called "pectic substances" in both the isotropic and the anisotropic layers. After prolonged treatment of sections in hot 12 per cent HCl the anisotropic layers stained faintly with ruthenium red, brightly with congo red. Since the isotropic layer did not stain it evidently was removed by the acid treatment. These results further indicate that the materials staining red with ruthenium red are pectic in nature.

The optical and chemical properties of the end walls of the vessel elements were the same before and after the lignification of the secondary longitudinal walls. The presence of lignin was determined by the Mäule test. At all times the end walls reacted negatively in the Mäule test, that is, they showed no lignification.

The doubly refractive layers of the end walls show optical and microchemical reactions similar to those of the not yet lignified longitudinal

[6] This procedure was suggested by Professor I. W. Bailey, Harvard University.

walls of the vessel elements and the primary walls of the adjacent cells. The anisotropy of these two layers evidently results from the presence of cellulose. They are here interpreted as the two primary walls of the two superposed vessel segments, and the isotropic layer is regarded as the intercellular substance, which is probably pectic in nature.

SUMMARY

The differentiating vessel elements of the herbaceous angiosperms here considered show intact end walls until the future vessel reaches its final diameter and develops secondary lignified layers on the longitudinal walls.

Two superposed vessel elements are separated from each other by two cellulose layers—the two primary walls—cemented together by isotropic intercellular substance.

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PLATES

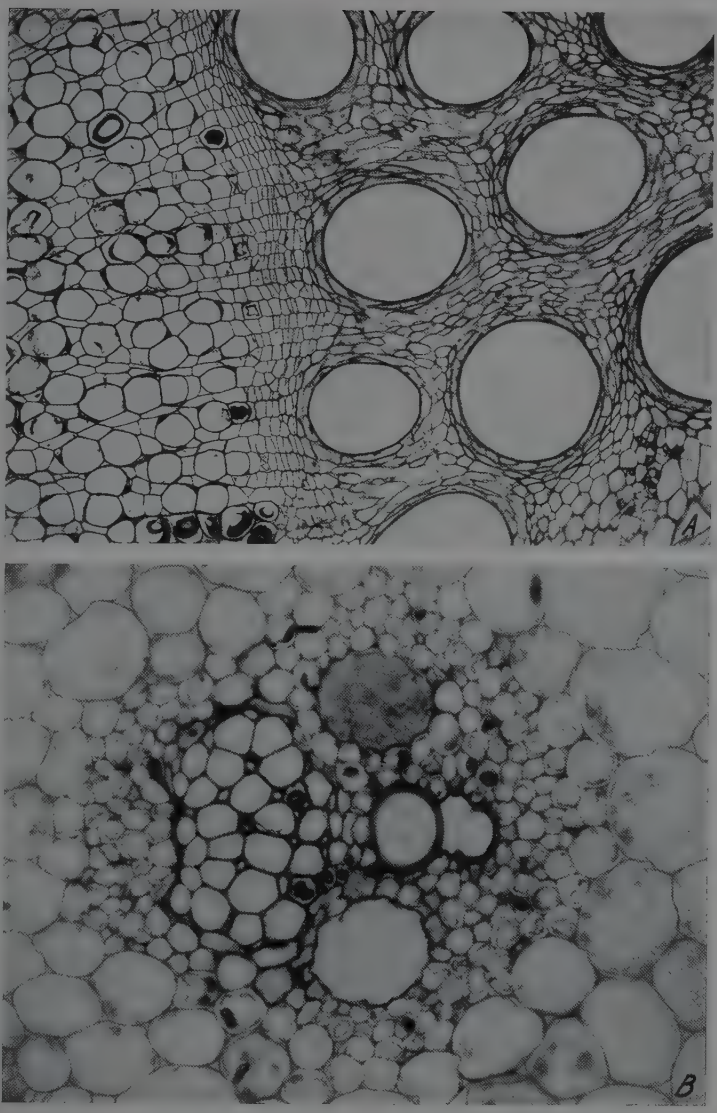

Plate 1.—*A*, Transverse section of part of a vascular bundle of *Cucurbita* showing phloem to the left, xylem to the right, and cambium between the xylem and phloem. (× 90.) *B*, Transverse section of a vascular bundle of *Zea* showing phloem to the left and xylem to the right. (× 400.) Further explanation in the text.

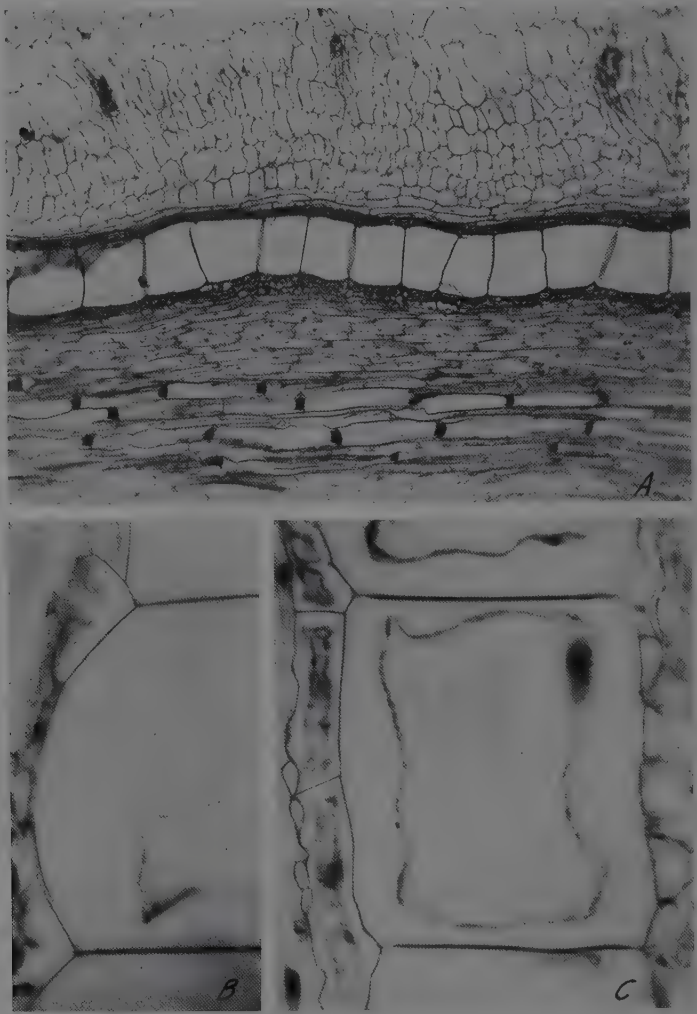

Plate 2.—Differentiating vessel elements of *Cucurbita: A*, longitudinal section of part of a vascular bundle showing phloem below and, above the phloem, a row of wide vessel segments with intact end walls (× 90); *B*, portion of a vessel segment with thickened end walls; *C*, entire vessel element with protoplast and thickened transverse walls. (*B* and *C*, × 540.)

Plate 3.—*A*, Longitudinal section of a portion of a *Zea* vessel. The end wall has been treated with zinc-chlor-iodide and mounted in the same solution. The transverse wall is bordered on the upper and lower sides by dark lines, which were stained blue in the original preparation, whereas the rest of the wall was stained yellow. (× 1200.) *B*, Longitudinal section of part of a pitted vessel of *Nicotiana;* a portion of the intact oblique end wall and the closing membranes of the bordered pits appear black. (× 540.) *C*, Longitudinal section of *Cucurbita* xylem showing the result of the tearing apart of parenchyma cells that occurred near an expanding vessel. (× 400.)

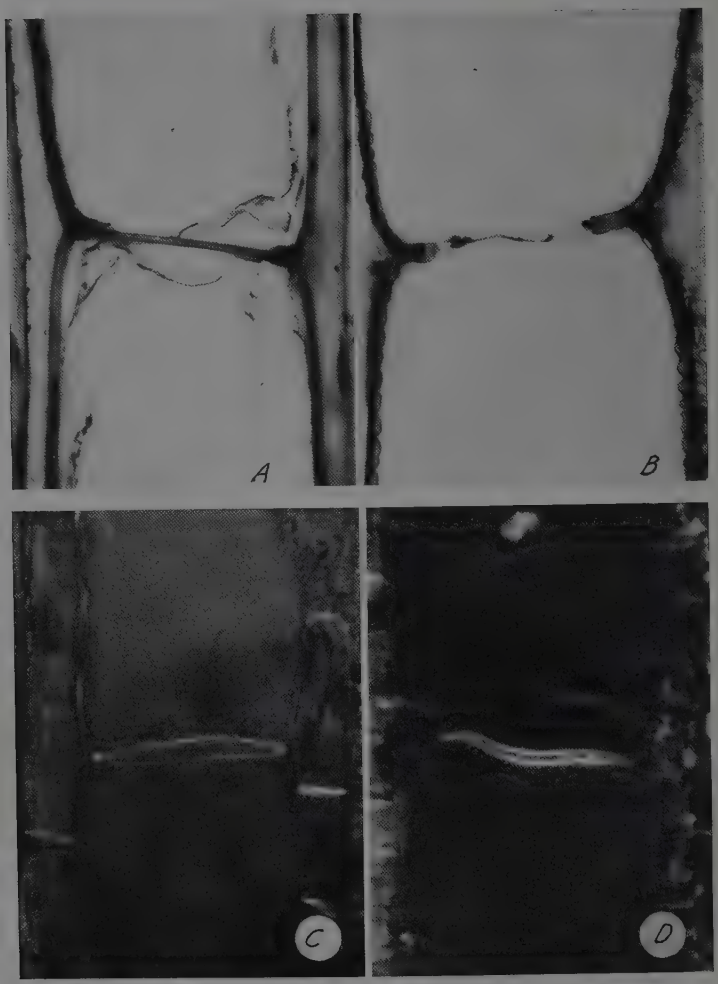

Plate 4. Longitudinal sections of fully expanded vessel segments of *Zea*: *A*, with secondary longitudinal walls and intact primary end wall; *B*, with disintegrating material in place of end wall. *C* and *D* show the appearance of the end wall mounted between crossed Nicol prisms: two peripheral anisotropic layers are separated from each other by isotropic material. The section in *C* was treated with hot water for 15 minutes; the one in *D* was stained with congo red; both were mounted in water. (All × 540.)

COMPARATIVE HISTOGENESIS OF VEGETATIVE AND FLORAL APICES IN AMYGDALUS COMMUNIS, WITH SPECIAL REFERENCE TO THE CARPEL

REID M. BROOKS

COMPARATIVE HISTOGENESIS OF VEGETATIVE AND FLORAL APICES IN AMYGDALUS COMMUNIS, WITH SPECIAL REFERENCE TO THE CARPEL[1]

REID M. BROOKS[2]

INTRODUCTION

THE INTERPRETATION of the carpel[3] as a foliar structure has been criticized both favorably and adversely. Evidence for and against Goethe's classical theory of metamorphosis in reference to the flower has been presented from a study of the vascular system of floral organs, paleobotanical material, and ontogeny. Thus far, however, no one has made a comparative histogenetic study of the mode of initiation of the foliage leaf and floral organs within the same species, together with an interpretation of the homologous or analogous nature of these structures.

Since, however, reproduction should be treated as a function of the entire plant, the reproductive shoot cannot be understood except in relation to the vegetative body (Arber, 1937).[4] As a background, accordingly, for the interpretation of the carpel, the vegetative apex and the production of foliage-leaf primordia have been studied, by the present author, within the same species in order to permit a comparison with the floral apex and initiation of floral organs, with particular emphasis on the carpel.

In attempting to compare the carpel and the vegetative leaf, the histogenetic method of attack attempts to do more than merely describe the expected anatomical differences between the two types of structures. This method seeks to explain the nature of the organs in terms of their origin from an initial cell or region and to show the distribution of growth in each. The general morphological development of an organ may be known; but the histological method associates these modes of growth and development with the localization, the type, and the duration of the different kinds of meristematic phenomena. At present there is an increasing interest in the morphogenetic significance of the early phases

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[3] In this paper, *carpel* refers not only to a single, simple, closed macrosporophyll, but also to each individual macrosporophyll unit of a compound pistil.

[4] See "Literature Cited" for complete data on citations, which are referred to in the text by author and date of publication.

of form development that results from the activity of primary meristems.

The research here reported is confined to *Amygdalus communis* L., horticultural variety Nonpareil, a commercial paper-shell almond. The purpose is two-fold: (1) to compare the development of the flower bud with that of the leaf bud, especially foliage-leaf initiation with the origin of floral organs; (2) to trace the development of the flower, particularly the initiation and early development of the carpel. The presentation of such evidence may suggest a new mode of attacking a problem of considerable morphological interest. The general applicability of the inferences drawn must await broad comparative studies of a similar character.

REVIEW OF LITERATURE

The idea of the homology of plant parts, especially leaf and flower, had been recognized before Goethe's time by Linnaeus (1759, cited by Lindley, 1832; 1760, cited by Lindley, 1832, and also by Schleiden, 1849) and by Caspar Friedrich Wolff in 1759 (cited by Bower, 1935, by Green, 1909, and by Sachs, 1890). According to Worsdell (1916), however, the views of Wolff and Goethe were probably founded on teratological data; evidence of the sort has been brought to bear upon this problem (cf. Goebel, 1895; Bancroft, 1935; and Kausik, 1938), but will not be discussed here. Goethe not only recognized these affinities but attempted to explain them in a theory of metamorphosis published in 1790 (at Gotha) in his *Versuch die Metamorphose der Pflanzen zu erklären.* According to Goethe the metamorphosis of plants is the phenomenon by which one and the same organ appears to us under numerous diverse forms. He called this original organ or prototype, whence arise all appendages of the stem, the "leaf" (*Blatt*), although the term never quite satisfied him and today causes much discussion because of its varied interpretations. Thus Goethe, in recognizing "the fertility which lies hidden in a leaf," developed, more or less abstractly, the idea that floral organs are homologous with foliage leaves. This *literal* interpretation of the theory is at present rather prevalent (Eames and MacDaniels, 1925; Hutchinson, 1926; Robbins and Rickett, 1929; Wilson and Haber, 1935; and Newman, 1936).

Goethe's theory of the foliar nature of the flower has been interpreted *figuratively* by Gray, A. Arber, and others (James and Clapham, 1935). Gray (1845) stated that the term *metamorphosis*, as applied to the floral organs, is figurative: foliage and floral leaves do not develop one from the other, although they may have the same underlying nature. This

same idea was expressed verbatim in the fifth edition of his *Botanical Textbook* (1871); yet, in another text of more recent date, he remarked that a pistil "consists of a leaf with its margins curved inwards till they meet and unite to form a closed cavity, the ovary, while the tip is prolonged to form the style and bear the stigma" (Gray, 1878). Recently A. Arber (1937) has discussed this question in detail, suggesting the term *parallelism* (within the development of the individual) in place of *equivalence, correspondence,* or even *homology,* whereby one compares the carpel to an infolded foliage leaf instead of calling it such a leaf.

Initiated by Goethe, the theory came to full fruition with A. P. de Candolle (1827, 1841), who placed it upon a much firmer and more definite basis and directed general attention to this conception. Basing his investigations upon external form-relations (as did Goethe), de Candolle (1841) considers the flower "an assemblage of several (usually four) verticils of leaves, variously transformed." Referring to the carpel he states, "Each carpel may be considered as a leaf folded longitudinally upon itself." Also, he gives many characteristics such as texture, color, presence of stomata and hairs, vascular system, and degeneration that aid in showing a similarity between carpel and leaf.

Brown (1840), a contemporary of de Candolle, in laying the foundation of floral anatomy and in using the microscope for the first time as mentioned in an earlier publication (1833) in such work, wrote: "The simple ovarium .... consists of the modification of a leaf folded inwards and united by its margins, which in most cases are the only parts of the organ producing ovula ....." To Lindley the metamorphosis of the organs is synonymous with the study of morphology (1832); further, "the ovarium itself is a convolute leaf, with its coats elongated into a style, and the extremity of its vascular system denuded under the form of a stigma ....." Braun (1851) also supported this theory of metamorphosis, interpreting the sporophylls as modifications of Goethe's hypothetical leaf, although he did not put forward a definite conception of this "ideal leaf." Thomas (1932) has remarked that present conceptions of the morphological relations of the higher plants, as expressed by such investigators as Velenovsky (1905) and Troll (1928), closely resemble those ideas noted by Goethe, Lindley, and de Candolle.

After this formal and abstract treatment of the problem (primarily from the organographical viewpoint), studies diverged along two general lines—morphology and physiology. The former point of view will be emphasized here, the physiological viewpoint being concerned primarily with the influence of internal and external factors on the structure of the shoot apex. As Klebs (1914) has shown experimentally, flower

formation is related to quantitative seasonal differences in the substances supplied to the growing apex. In *Scrophularia,* Schmidt (1924) found that there are more meristematic layers at the apex at the time of flower formation than before this stage. Priestley (1929) discovered that at the time of flower formation, the meristematic layers at the apex occur at a greater depth because of a greater thickness of cell walls and a consequent increased water supply through the walls to the protoplasts.

In morphology the different interpretations of the carpel may be based upon three main trends of thought—namely, on the vascular anatomy of the flower, on paleobotany, and on ontogeny. Necessarily, in any comparative morphological investigation, the facts arising from these sources cannot be divorced from one another in a true analysis of a structure such as the carpel; all are related to phylogeny. No doubt the "full problem of morphology is," as Bower (1908) noted three decades ago, "to explain . . . . how in the past plants came to be such as we now see them."

Brown (1822) had pointed the way in research on the vascular system when he wrote: "In the ovarium . . . . the vascularity, compared with that of the leaf, is in general rather modified than diminished; the principal vessels occupying the margins or lines of production, and giving off branches toward the axis (midrib), whose vascularity is frequently reduced." Payer (1857, cited by Hunt, 1937) asserted that the carpel "is formed by an appendicular part, the carpellary leaf, inserted by its base on the two branches of a bifurcated axis which carríes the ovules." Van Tieghem (1868) saw in the vascular system of the flower a main point on which the interpretation of the carpel may be based, and in 1875 (cited by Hunt, 1937) he "established the appendicular nature of the entire carpel (in the sense that the leaf is appendicular)." Earlier, though, Cave (1869) had stated: "La majorité des savants considèrent ces organnes (les carpelles) comme exclusivement appendiculaires." Eichler's (1875) contribution was his *Blüthendiagramme* with a discussion of floral morphology from the viewpoint of the evolutionist. Henslow (1891), greatly influenced by the writings of van Tieghem, believed that the vascular strands of the floral whorls are fundamentally alike and that this similarity enabled the different parts of the flower to interchange their structure and function. E. A. N. Arber and Parkin (1907), following the dictum of Goethe, regarded the carpel as a fertile leaf, more or less modified.

New impetus, which has carried to the present time, was given to research in floral morphology in 1923 when Saunders reopened this subject by publishing a theory of carpel polymorphism. She (1937) in-

terprets the gynoecium in accordance with the view that in true apocarpy the individual ovary is formed of a single carpel, which arises as a separate structure, but that in syncarpous and pseudo-apocarpous forms it is composed of two kinds of carpels, sterile and fertile. She (1931) conceives of the polymorphism of carpels as arising simultaneously with syncarpy. Further, these two kinds of carpels differ markedly in form as well as function and compose two alternating whorls. Authors (among them A. Arber, 1933; Eames and Wilson, 1928; but cf. Wilson, 1937, noted below) preferring the classical interpretation of the flower have held that the vigor of vascular development has shifted from the midrib to the marginal bundles and that "normally, in the majority of Angiosperm families, a carpel receives three traces, a dorsal or midrib bundle and two ventral or marginal bundles" (Eames and Wilson, 1928). But Saunders (1934), holding to the theory of carpel polymorphism, views "the *vigorous marginal* veins as the *main* bundles of intervening consolidated carpels" and declares that "the vascular supply of the valve carpel is *in its origin* precisely similar to that of the members of the other floral whorls ..... Whether a carpel has one trace or three *at the level of exsertion* is merely a question of whether the midrib happens to give off its first laterals before or after exsertion."

Eames (1931), using the vascular anatomy of flowers as Saunders has done, concluded that "the theory of polymorphism is clearly unsound." For him the flower is a specialized stem, all the floral organs being homologues of leaves; polymorphism has erred in interpreting the anatomy of the flower, especially as regards the carpellary vascular system. From a study of the vascular supply to sepal, petal, stamen, but not carpel, Wilson (1937) concludes: "The modern stamen is not homologous with the entire leaf, as stated under the classical theory of the nature of the sporophylls of the flower; it is rather homologous with only a part of a leaf, and the term sporophyll may no longer be applied." As concerns the stamen, therefore, he has modified his viewpoint on the classical interpretation of the flower as noted above (Eames and Wilson, 1928).

Significant facts unearthed by paleobotany have assisted in determining the nature of the carpel even though it be static, rather than growing and changing in form and structure. According to Thomas (1934) the angiospermic flower is not the homologue of a vegetative bud. From studies on the reproductive bodies of the Caytoniales, a Lower Jurassic group of fossil plants, he concluded that certain structures originally fertile have been sterilized. Further, he states, this transition is much more likely to have taken place in almost all the higher plants than is the derivation of anthers, carpels, and ovules from expanded foliar

structures with fertile edges (Thomas, 1931; cf. Bancroft, 1935). According to this interpretation, expressed by Thomas (1936), the carpel of a flowering plant is composed of two fused cupules rather than of a single foliar structure.

Parallel with these studies on paleobotany and floral vascular systems, there has developed an extensive literature dealing with the comparative anatomy of the various foliar organs in angiosperms. Contributions, however, to the problem of foliar histogenesis and floral ontogeny have been made only recently. No doubt Schuepp (1926) has made one of the most important of these in his review of meristems and their relation to growth processes. This work has been greatly furthered by Foster (in *Carya Buckleyi*, 1935*a*, 1935*b*, and *Rhododendron* spp., 1937*a*, 1937*b*) by studying histologically the shoot apex and the development of various foliar organs, and by discovering the differences between cataphyll and foliage-leaf primordia. Opposing the ontogenetic method is the "typological method" advanced by Troll (1935), whereby the comparative study of a wide range of adult forms is considered far more important than studies pertaining to the ontogeny of such structures. Conclusions based on observations of mature structures cannot be depended upon alone unless accompanied by and correlated to comparative results concerning the developmental phases of the organs themselves (Cross, 1937*b*).

Of the recent interpretations of the carpel based on ontogeny, three important ones have been promulgated by Grégoire, Newman, and J. McLean Thompson. Grégoire (1931) was among the first to investigate histogenetically the floral and vegetative apices of angiospermous plants and review comprehensively (1938) the comparative nature of these apices. Through these earlier studies he found two essential differences between the development of the flower and of the vegetative shoot.

In the structure of the vegetative "cone" Grégoire finds that a "tunica," consisting of several self-perpetuating cell layers, envelops an inner mass of cells, the "corpus," furnished with an initial region that produces growth in length. The floral apex, however, possessing no particular initial region, has only a meristematic "hood" or "mantle" covering a raised parenchymatous mass, which cannot become longer. Thus the floral meristem contains no tissue corresponding to the tunica and corpus of the vegetative cone (Grégoire, 1935). A corollary of this conception forms another point of difference between floral and vegetative shoots; the floral apex as thus constituted gives rise only to the petals, stamens, and pistil; "the sepals begin development *as leaves on a vegetative cone*." Only after having produced the sepals does this vegetative

cone modify its divisions and its parenchymatous differentiation in such a manner as to become a floral axis.

From these observations that the meristematic origin of a carpel is not the same as that of a leaf, Grégoire (1931) reaches a conclusion opposed to the classical theory: *"Carpels do not represent modified leaves, but are organs 'sui generis,' without homology among organs of vegetative equipment."* Also, the dimensions of the apex may influence the type of structure to be produced, for he states that if the vegetative cone were larger, it apparently would have become a carpel itself. Unfortunately, in this earlier work, he compared the vegetative cone of one genus with the floral apex of another genus in securing and interpreting his results. This difficulty has been overcome to a certain extent in his most recent paper (Grégoire, 1938), whereby, at least in some instances, he figures and discusses the two types of apices in the same species (for example, *Aconitum napellus*). In this last paper Grégoire has emphasized further, by discussion and numerous illustrations, his original conception of the difference between vegetative and reproductive apices. No tunica is found in the floral apex corresponding to that in the vegetative cone; it is replaced by a homogeneous meristematic mantle, which represents "á lui seule, *tout le meristeme* du sommet floral" (Grégoire, 1938). Because of its innate structure this floral summit cannot increase its axis: it is a determinate structure. Thus the meristematic mantle is but a simple producer of the floral organs alone; the floral apex and the vegetative cone consequently belong to two entirely separate and distinct morphological categories.

Newman (1936), on the other hand, surveying the primordia of *Acacia longifolia* and *A. suaveolens*, favors the classical interpretation of the carpel as a modified fertile leaf; specifically he concludes that the fruit of these species, a legume, is a single, folded, foliar structure. He is among the first, if not the first, to describe floral meristems and the initiation of floral organs, especially of the carpel, in a truly ontogenetic manner, even though he based his descriptions on the generalized aspect of apical meristems and on their activity rather than on precise demarcation of tissue-areas. According to him there is no fundamental difference between vegetative and floral apices; and therefore the legume is initiated in the same manner as the other parts of the flower *and of vegetative leaves*, the ovules being found on the margins of the lamina of the legume. Newman's knowledge of the vegetative apex is obtained, however, only from the recent literature of other authors; he gives no information, either in text or figures, on the shoot apex or on the origin of the phyllode of *Acacia*. Yet he compares the carpel with the vegetative leaf

from the histological viewpoint, concluding that "there is no reason for doubting that the legumes of *Acacia longifolia* and *Acacia suaveolens* are of foliar nature, and that it is reasonable to interpret the flower of these species as modified leafy shoots." The carpel is thus homologous with foliar structures—a totally different conclusion from the one Grégoire reached by following virtually similar histological methods and also from the one J. McLean Thompson reached by using the same species of *Acacia* and similar histological methods.

J. McLean Thompson (1934, 1936*a*, 1937) conceives of the flower as a heterosporous sporogenous axis, with limitation of growth in one dominant direction and of continued growth in others, leading to the creation of one or a series of fertile style-bearing structures of axial origin, on which ovules may be formed. Although this sporogenous axis alone is essential to flowering, its base is sterile, marking the transition from the vegetative body to the spore-bearing organ. Bracts, bracteoles, and sepals are products of this sterile base. The lower portion of the sporogenous tissue is potential microsporangium, of which the lower emergences from this area are commonly sterilized and mature as either petals or staminodes. Further, says Thompson, if toral growth is dominant over apical growth from an early stage of development, the maturing axis is cup-shaped, lined with potential sporogenous tissue; and an inferior ovary is thus initiated. If, however, apical growth predominates, a flower with an hypogynous ovary results. Concerning the ontogenetic approach to the study of the morphology of the flower, Thompson (1934) states: "On the present view ontogenetic study of flowering is the only form of morphological enquiry which needs no apology. It is the basis of all comparison."

After Newman (1936) had published his paper on *Acacia*, J. McLean Thompson (1936*b*) wrote an article using the same species as did Newman but completely refuting Newman's conclusions. Instead of considering the legume of these species of *Acacia* as foliar in nature and the flower as a modified leafy shoot, Thompson believes: "There are no grounds for either of the above conclusions. They are merely in conformity with an historical interpretation which is not in accord with established facts. The carpellary theory of gynoecial construction has been a veil obscuring the true issues. Spore-production is the only fundamental feature in flowering, and the floral apex, whatever be its final form, is the seat of origin of megaspores and is the gynoecium."

Contributions in floral histology have been made in other lines of endeavor as well as in the realm of "pure botany." In the field of horticulture particularly, numerous papers such as those of Bradford

(1915), Tufts and Morrow (1925), Abbott (1935), Bonnett (1935), Aaron (1936), Winkler and Shemsettin (1937), and Barnard (1938) have reported the formation and differentiation of flower buds, especially as regards the time at which floral primordia are produced, and have attempted to correlate environmental conditions to this production. Unfortunately for the present problem, these investigations do not deal with the ontogenetical aspects of floral apices.

Judging from the review of literature pertaining to the interpretation of the carpel, the eventual clarification of this complex problem of the carpel and its developmental history must involve some reconciliation of all lines of attack—that is, a judicial application of data of ontogeny, vascular anatomy, paleobotany, and comparative morphology. At present this reconciliation is impossible because of the incomplete evidence, particularly with respect to the comparative histogenesis of the development of the vegetative and floral shoots. The present paper attempts to throw light on this aspect of the problem.

Investigations of the almond have been concerned, for the most part, with horticultural problems, whereas few have studied the histology of the almond flower and fruit. Brief reports have been made on certain of these structural features. Pease (1930) and Young (1912) have discussed the histological features of the almond seed coats. Campbell (1915) has briefly discussed the relation of stamens to insects in pollination of the species. Bonne (1928, cited by Ragland, 1934) has described the vascular system of the pedicel and receptacle of both the almond and the peach. Cave (1869) has given certain features of the vascular and anatomical structure of the fruit. The most comprehensive treatise on the histology of the almond was that wherein Garcin (1890) discussed minutely the tissues concerned in the mature fruit rather than the buds or flowers.

MATERIALS AND METHODS

The material of *Amygdalus communis* L., horticultural variety Nonpareil, used in this investigation was collected from a single tree growing on the University of California Farm, Davis. Leaf buds were collected semiweekly from their time of formation through differentiation of the floral organs. Flower buds, flowers, and fruits were collected also at semiweekly intervals throughout the growing seasons of 1936 and 1937. Spurs with the buds were brought to the laboratory, where, in the case of the mature buds, the outer foliar structures were removed and the buds were placed directly either in Navaschin's fluid (Derman's Harvard modifica-

tion)[5] or in a formalin-acetic-alcohol fluid[6] for killing and fixing. The material was evacuated while in the killing reagent. Flowers and fruit were preserved in the formalin-acetic-alcohol fluid.

The usual alcohol-xylol-paraffin schedule was followed, serial sections being cut 6–10 microns in thickness. Transverse serial sections were cut from the base upwards. A tannic acid-iron chloride method (Foster, 1934) and a modified safranin-fast green FCF staining schedule (Moore, 1936) were used. As the former schedule showed well the cytological features of the meristematic region of buds together with cellular detail of carpel development, it was relied upon for the greater part of the work.

All drawings were made with the aid of a microprojector, using an oil-immersion lens wherever cell structure is shown. Primordia were measured (1) by using an ocular micrometer in conjunction with a calibrated glass slide, (2) by measuring the drawing directly with a slide micrometer in the microprojector, and (3) by multiplying the number of serial sections by their thickness in microns.

GENERAL MORPHOLOGY OF THE SHOOT SYSTEM

The majority of the almond flower buds are borne laterally on short shoots (spurs) of the past season (fig. 1, *A* and *B*), only a few occurring on comparatively long shoots of the past season (one-year-old shoots, fig. 1, *D*). To be certain of securing flower buds they were selected only from spurs, where they appear as a rosette of axillary buds around a single terminal leaf bud. Flower buds on a spur and on a long shoot are distributed in a similar manner. On a spur, flower buds are produced only at the nodes nearest the terminal bud (which is always vegetative). The number of flower buds per spur varied from one to six, with an average of three in the rosette. On a long shoot (fig. 1, *B* and *D*), especially on one-year-old shoots 6 inches or more in length, most of the flower buds occur near the apex, involving as many nodes as there are on an individual spur, but of course not arranged in a rosette. Below these three-to-six apical flower buds, on the remainder of the long shoot, flower and leaf buds are arranged in no definite sequence at the nodes, although the number of leaf buds predominates. Thus, below the terminal leaf bud of both spur and long shoot, the apical one-to-six flower buds are usually distributed in a similar manner. Gourley (1938) has noted the existing confusion regarding the terms *flower bud* and *fruit bud*. Since abundant

[5] Solution A: 1.5 grams chromic acid, 12 cc glacial acetic acid, and 86.5 cc water. Solution B: 50 cc commercial (40 per cent) formalin, and 50 cc water.

[6] 5 cc formalin, 5 cc glacial acetic acid, and 90 cc 70 per cent ethyl alcohol.

Fig. 1.—*A*, Four-year-old shoot (June 8, 1938) showing the growth habit. The upper fruit shows the ventral suture and remnant of the style; the lower fruits show the dorsal side. *B*, Two-year-old shoot (September 14, 1938) showing the formation of the spur type of growth; a long shoot was produced also during the current season. *C*, Four-year-old shoot (September 14, 1938), farther advanced than that in *B*. Leaves or leaf blades have been removed to show the rosette of buds on a spur. *D*, Current shoot (June 8, 1938) showing growth habit, axillary, and terminal buds. *E*, Diagram of the almond flower, showing the sympetalous calyx, the arrangement of the corolla, the three whorls of stamens, and the unilocular carpel containing two ovules. (*A–D*, ×⅕.)

flower production is no guarantee of fruit production, *flower bud* is considered the better term and is used in this paper (cf. Loehwing, 1938).

The leaf bud is triangular in longitudinal section, the basal portion being extremely woody—much more so than in the flower bud. Flower buds are longer, narrower, and more pointed than the other type. Both kinds are covered with deep-brown cataphylls (bud scales); those of flower buds, however, bear a distinct whitish fringe along the margin of each scale. The cataphylls on both bud types exhibit imbricated vernations, those of the leaf bud being much tighter.

Noticeable swelling of the flower buds began nine weeks before the first flowers opened on the tree and continued during that period of time even though temperatures ranged down to 14° F during January of one year (1937). Six weeks after swelling began, the inner bud scales were a light green and the calyx a darker green, although none of the structures was exposed to light; the scales were removed very easily for fixation. Two weeks later the petals were pink; but not until four days later did they protrude through the scales.

Leaf buds began to enlarge some eight weeks after the flower buds. The tree was in full bloom (fig. 11, *A*) about five days before the leaf buds began to open. Within ten days after bloom, most of the petals had fallen.

The form or design of the flower is shown in the floral formula, $\frac{Ca^5Co^5S^{30}P^1}{Dr}$. The general structure of this perigynous flower is shown in a floral diagram (fig. 1, *E*). Ten stamens are borne in each of the three whorls. The outer whorl is arranged in five antepetalous pairs; the innermost whorl in five antesepalous pairs. The stamens of the center whorl are located opposite each pair of stamens of the outer rows: each central filament is subtended by a U-shaped structure, the nectary. Viewed differently, all stamens except five antepetalous stamens of the second whorl are opposite the calyx lobes, five in each group. Each of the thirty stamens is located on a different radius. The stamens of the innermost (adaxial row) are shorter than the others, being 6.0 mm long; those of the central whorl range from 7.5 to 10.0 mm; those of the outer (abaxial row) from 10.0 to 12.0 mm (fig. 11, *A*).

The petals of this cultivated variety exhibit three types of estivation, of which the most common is illustrated in figure 1, *E*: several authors, among them Small (1921), term this "the cochlear descending imbricate type." Petals are borne on the rim of the floral tube between the calyx lobes. The single carpel contains two ovules, of which only one usually matures (fig. 11, *B*).

After fertilization the calyx persists for some four weeks; during the

latter part of this time an abscission layer forms on the receptacle near the base of the carpel. Growth of the fruit ruptures the floral tube, which then falls off, bearing the withered stamens. After elongation of the peduncle, cataphylls fall about ten days after full bloom. The style withers shortly after fertilization, although it may persist almost until fruit maturity. Leaves remain on the tree until November or December under normal conditions.

SUCCESSIVE STAGES IN THE HISTOGENESIS OF THE LEAF BUD

Although the flower may be compared to a vegetative shoot, the two structures show important differences. These dissimilarities, together with the likenesses, have been aptly pointed out by A. Arber (1933, 1937) for the general form of the organs involved. To view further the homologies and analogies of the vegetative and floral axes, the histological and (as far as possible) the cytological aspects of the apices of both leaf and flower buds have been investigated. The early ontogenetic development of the leaf bud will be described first.

Organization of the Apex.—Organization is concerned with the architecture of the apex[7] and with the precise demarcation of the tissue areas that give rise to the cataphylls and foliage leaves in the bud. The term *histogenesis* has often been used to refer exclusively to cell destiny, but such usage is not correct. Problems of growth in surface and volume in a meristem are quite apart from the destiny or fate of the cells themselves; thus arise two viewpoints, exemplified by Hanstein and by Schmidt.

Hanstein (1868) was concerned with the destiny of cells. He divided the meristem into three different histogens: the "dermatogen," which produced the epidermis; the "plerome," which produced the central cylinder; and the "periblem," a region lying between these, which usually formed the cortex and sometimes the pericycle and vascular tissue also. But Hanstein's divisions have been discarded by several recent workers —Schmidt (1924), Rösler (1928), Zimmermann (1928), Kühl (1933), Foster (1935*a*), and Cross (1936). The publications of de Bary (1884), Schoute (1902), and Strasburger (1908) show that the Hanstein conception is not applicable to the apices of all plants. Foster (1935*a*), Cross (1936), and others (Eames and MacDaniels, 1925) have demonstrated

[7] The term *apex* seems preferable to the ambiguous though common *growing point*. Considering the complex character of *growth* and the impossibility of localizing it to a point, it seems preferable to substitute the expressions *vegetative apex* and *floral apex*, respectively, in speaking of the entire embryonic terminal region of a leafy and a floral shoot.

that the three classic histogens, even when present, have little morphological significance.

Thus there has arisen another viewpoint of histogenesis, as expressed above, which is concerned primarily with the genesis and growth of cells in the meristem region itself. Following this trend, Schmidt (1924) proposed the terms *tunica* and *corpus* as substitutes for those used by Hanstein; Lund (1872) expressed a similar conception some fifty years earlier in his idea of perinome and pycnome (Foster, 1936*b*, 1937*a*). In the present paper *tunica* and *corpus* will be used. Schmidt defined them as follows: "The tunica is the apical external layer or layers of cells which do not ordinarily divide in the periclinal plane except during leaf or bud initiation. The corpus includes all the remaining internal tissue in which divisions commonly occur in diverse planes." As Foster (1935*a*) has reported occasional periclinal divisions in the tunica of *Carya Buckleyi,* evidently these zones are not sharply demarcated. In this paper Foster has discussed the general applicability and validity of Schmidt's conception: namely, it is applicable to certain cases in which there is no distinction between periblem and plerome; by avoiding the implication that cortex and stele are always derived from independent histogens, it allows for the wide variability known to occur; and finally, it unites Hanstein's dermatogen and periblem under the collective term of tunica.

The apex during cataphyll and leaf formation exhibits a broad, domelike, slightly convex appearance in median longitudinal section, its width being about 125 microns. The apical meristematic region of the leaf bud consists of a distinct four-layered tunica and an extensive corpus (fig. 2, *D*; plate 1). This condition may be observed at all times in differentiation in the almond (fig. 2, *A, B,* and *C*; plate 1, *B*). Cross (1937*b*), however, noted in *Viburnum rufidulum* that the four-layered condition was easily shown only at the end of the production of an organ, when the apex had attained its greatest surface area. For convenience in description, the layers of the tunica are numbered inwardly and designated as *t-1, t-2, t-3,* and *t-4.*

The outer row of cells of the tunica, *t-1,* always forms a definite and discrete layer over the apex, keeping its identity in the developing primordium. This layer corresponds to the dermatogen of Hanstein and is characterized solely by anticlinal divisions in the almond; it forms the epidermis of both the foliar and floral organs and of the stem. The subepidermal region, consisting of *t-2, t-3,* and *t-4,* constitutes an individual zone over the entire apex, dividing periclinally only during foliar initiation (fig. 3, *A*). This cell division is limited, however, to that region from

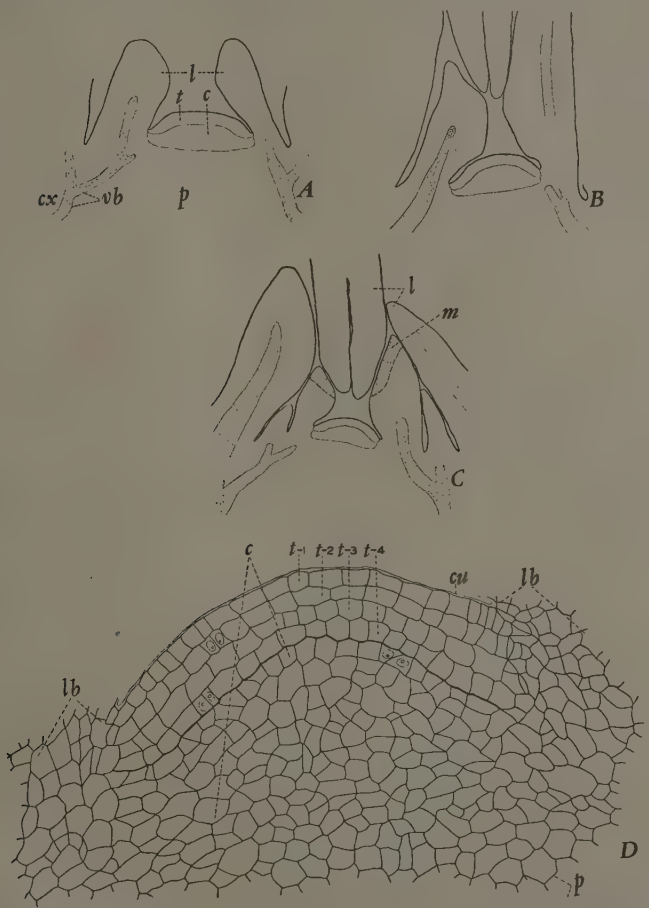

Fig. 2.—*A–C*, Outlines of median longitudinal sections of the apices of leaf buds collected, respectively, May 18, July 11, and December 7, 1936. *D*, Apex of the leaf bud showing the four cell layers of the tunica and the large corpus, and the mode of cell division in each region. Details are: *c*, corpus; *cu*, cuticle; *cx*, cortex; *l*, leaf; *lb*, leaf base; *m*, meristematic zone; *p*, pith; *t*, tunica; *t-1*, *t-2*, *t-3*, *t-4*, rows of cells in the tunica; *vb*, vascular bundle. The dotted lines at the apex in *A–C* demarcate the tunica and corpus regions in this and all similar figures which follow. The heavy line in *D* delimits the tunica and corpus regions of the apex in this and all similar figures which follow. The nuclei indicate the type of division in the various regions. (*A–C*, × 82; *D*, × 436.)

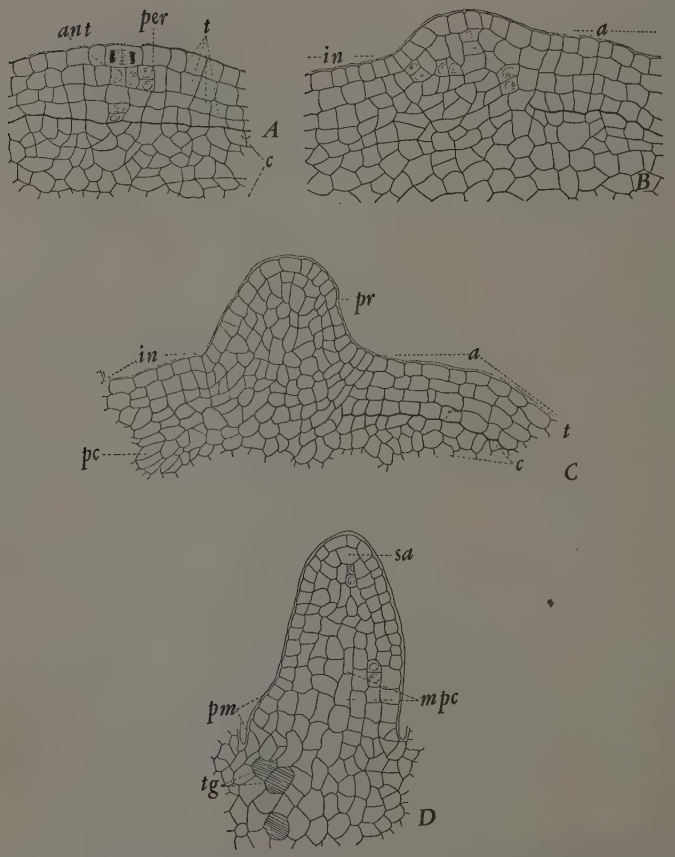

Fig. 3.—*A*, Side of the apex of a leaf bud, showing initiation of a leaf by periclinal divisions in the tunica region. *B*, Primordium of a leaf shortly after the stage shown in *A*. The tunica has now lost its identity in this initiating region. *C*, A developing leaf primordium, showing its relation to the apex and the internode. The tunica and corpus are clearly demarcated in the apex which has not given rise to the leaf. *D*, Leaf primordium in an advanced stage of development, 90 microns in height. Procambium and petiolar-midrib region are being initiated. Details are: *a*, apex; *ant*, anticlinal division; *c*, corpus; *in*, internode; *mpc*, median procambial strand; *pc*, procambium; *per*, periclinal division; *pm*, petiolar-midrib region; *pr*, primordium; *sa*, subapical cell; *t*, tunica; *tg*, tannin globules. (*A* and *B*, × 465; *C* and *D*, × 372.)

which the primordium arises, not being found below the immediate summit of the apex. The corpus tissue extends vertically for some thirteen to fifteen layers of cells, until the pith of the stem is reached. Laterally the corpus extends to the toral procambial strands. No vascular strands have appeared as yet in this region (fig. 2).

Since bud scales of the leaf and flower buds are morphologically similar, no attempt has been made to compare cataphyll initiation in the two bud types.

Mode of Initiation of the Foliage Leaf.—Initiation of the leaf is first evidenced by rapid anticlinal divisions in the surface layer, *t-1;* as few as two or three cells may start this initiation (figs. 2, *D,* and 3, *A*). Vertical elongation produces a perceptible swelling on the surface of the apex, followed immediately by periclinal divisions in two or more cells of *t-2* which then elongate vertically also, giving the characteristic raised portion of a primordium. Division then ensues in *t-3-4,* so that shortly the identity of the subepidermal cells (*t-2-3-4*) as part of the tunica is lost (fig. 3, *B*). Cells of the corpus do not divide at this time, for the highly meristematic region extends only through the tunica at this stage of development; hence the corpus does not enter into leaf initiation at this point. In the primordium itself, after several divisions have taken place, there is no evidence of a discrete tunica and corpus (fig. 3, *B* and *C*). It may be noted, therefore, as Foster (1936*a*) did for *Carya Buckleyi* var. *arkansana* and Cross (1937*a*) for *Morus alba,* that there is no clear demarcation between the derivatives of the inner tunica layer in the apical region of a primordium. Thus the tunica does not maintain its individuality in the leaf, except for the epidermal layer.

At a height of 90 microns the foliar primordium differentiates a median procambial strand by periclinal divisions in the cells of the central region. These cells elongate parallel to the length of the primordium so that a distinct procambial region is delimited some 6 to 15 cells below the apex, forming an area 1 to 4 cells in length (fig. 3, *D*). It then develops both acropetally and basipetally until it joins the vascular system of the stem axis. Evidence of differentiation in this median procambial strand is first noted considerably after the vascular system is continuous into this axis.

Viewed transversely instead of longitudinally, the young leaf exhibits no marginal meristem—that is, the surface divides anticlinally only, preserving its identity; nor is there indication of any apical meristem cell, although the tip of the leaf blade is characteristically more meristematic than the remainder of the cells (fig. 2, *C*). A large subapical initial has been observed several times, however, directly below the apical epider-

mis; from this initial, apparently, arise all the rest of the cells of the mesophyll tissue (fig. 3, *D*). The subapical cell, when seen in longitudinal sections, cuts off cells periclinally, which then become greatly elongated as the base is approached. How long this cell functions was not determined.

The young leaf increases in length by divisions throughout its entirety until a height of 90 to 120 microns is reached. Then the cells of the adaxial surface become highly meristematic, producing a slight swelling (Cross, 1937*b* and 1938); this is the early differentiation of the petiolar-midrib region (figs. 2, *B*, and 3, *D*). Differentiation of the embryonic leaf into the characteristic mesophyll tissue of the mature leaf takes place exceedingly late—often as late as twenty days before the bud opens.

At the time of procambial formation, scattered cells in the hypodermal layers of the embryonic leaf begin to fill with what appear to be minute globules of tannin, which later coalesce into a solid mass completely filling the cell (fig. 3, *D*). Also, at a somewhat later stage, several tannin-filled cells are around the median vascular strand. As the leaf grows, cells containing tannin are more prevalent around the vascular elements than elsewhere, until, in a leaf several months old, as viewed transversely, the midrib area is characterized principally by such cells surrounding a small vascular bundle. In addition, at this stage more of the subepidermal cells of the lamina are filled with the red globules as stained by the safranin; but they are still relatively few. In the cataphyll, however, there is a preponderance of these tannin cells throughout its entirety; they do not seem to be localized as in the foliage leaf.

Throughout the growing period of the leaf bud, the meristematic region of the apex maintains its characteristic four-layered tunica and central corpus. The corpus adds cells basipetally to the stem region, as evidenced by the proportionate increase in larger vacuolated cells with thicker cell walls found below the dense, closely packed cells of the corpus. Here the corpus gives rise to the pith, procambium, and part of the cortex. Corpus cells divide anticlinally, periclinally, and obliquely in order to keep pace with the ever expanding apex as it continually gives rise to additional primordia.

Unicellular epidermal hairs arise early on the foliage leaf, soon becoming vacuolated and enormously long; these persist during the life of the organ. No druses are present except those found in the cataphylls.

Specific differences between foliage leaf and cataphyll initiation are difficult to determine because their early formative periods are essentially similar; but characteristics have been found that aid in delineating the two types of structures. The embryonic leaf is characterized by the

development of a median procambial strand early in the primordial stage when the primordium is less than 100 microns high; this strand develops at a height of 175 microns in the cataphyll. The foliage leaf shows less rapid and less extensive cell vacuolization during the early embryonic period than does the scale leaf (Foster, 1931) and is more meristematic in its staining reaction. In the cataphyll, many cells contain tannin, others contain druses; but in the foliar structure, few cells are filled with tannin, and none with druses. Judging from the present study, leaf differentiation, as apart from bud-scale initiation, begins shortly after the formation of the bud. True leaves were present in the leaf bud on May 4, 1936; these would unfold the following February (in 1937).

SUCCESSIVE STAGES IN THE HISTOGENESIS OF THE FLOWER BUD

In the entire history of flower-bud development, structures appear and differentiate in definite phases or cycles; each phase during which a certain structure arises may be termed a "stage." Floral histogenesis, as noted here, will be described as a series of successive stages. Such histogenetic development is concerned with the production of bud scales by the promeristem before differentiation of floral parts; with the change in form of the primordial meristem at the time of sepal differentiation; and finally with the acropetal succession of calyx, corolla, androecium, and gynoecium.

Stage 1, Ontogeny of the Apex up to Floral Differentiation.—The earliest phase in the history of the apex is distinguished by the characteristic structure and shape of the meristematic apex and by the production of cataphylls. At the time the flower bud is initiated, the surface of the apex covers an exceedingly small area (fig. 4, *A*) in comparison with that of the leaf bud (fig. 2, *A*) in a similar stage of development, for the width of the flower-bud apex between the bud-scale primordia is only 50 microns, whereas the leaf-bud type is 150 microns, or three times as great in width. The apices from which figures 2, *A*, and 4, *A*, were drawn are from serial sections of the same spur, in which one bud was terminal, the other lateral. The width of this floral apex, however, becomes progressively greater as more scales are produced, until, several weeks later, a width is reached (fig. 4, *B*) comparable with that of the leaf apex.

The summit of the floral apex shortly after initiation is but slightly rounded, extending only about the width of 1 to 3 cells above a line drawn between the bases of two opposite cataphyll primordia. It can hardly be

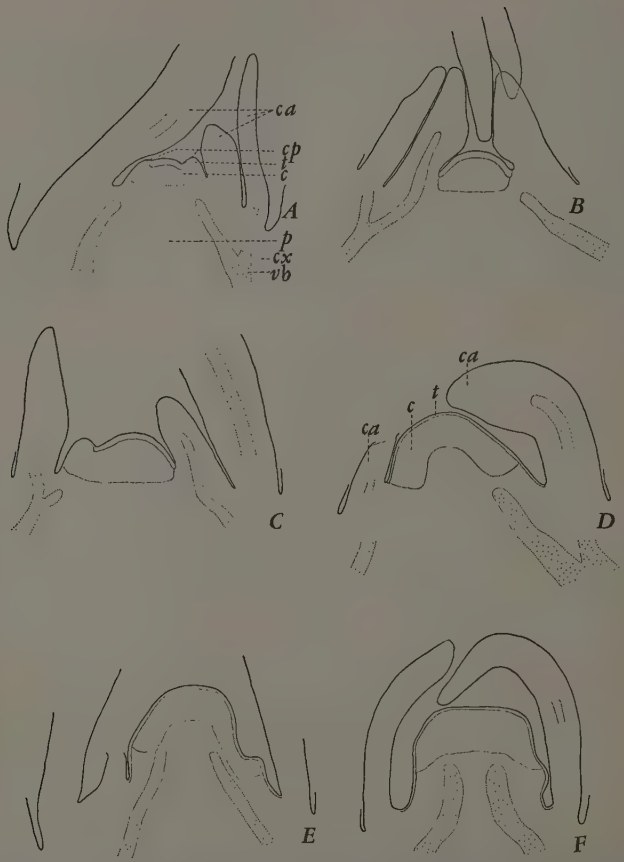

Fig. 4.—*A*, Very young flower bud (May 18) showing apical region, formation of cataphylls, and relation of tunica and corpus. *B*, Apex of flower bud (July 16) showing the type of growth in the developing cataphylls. *C*, Apex of flower bud (August 3) showing the origin of a cataphyll three weeks prior to the transitional stage. A two-layered tunica is present. *D*, Apex of flower bud (August 21) showing the transition from the vegetative type to the floral type of apex. The tunica is now uniseriate. *E*, Apex of flower bud (August 27) showing an advanced stage in the transition to the floral type of apex. *F*, Apical region of the flower bud (August 31) in the transition stage, just prior to sepal initiation. This stage shows the axis with a broad flat plateau and nearly vertical sides. The procambium is developing in the lower part of this region. Details are: *c*, corpus; *ca*, cataphyll; *cp*, cataphyll primordium; *cx*, cortex; *p*, pith; *t*, tunica; *vb*, vascular bundle. (All × 82.)

termed dome-shaped, like the leaf bud on the same date; yet before long the apex does begin to assume this shape. Thenceforth the apex becomes more convex, being higher above the bases of the bud scales, until it begins to assume the form and structure peculiar to the "transition" apex of stage 2. The floral apex during stage 1 is, then, much more pronouncedly dome-shaped than that of the vegetative apex (fig. 2, *C*, and 4, *B*; plates 1 and 2, *A*).

In contradistinction to the four-layered tunica of a foliar bud, a distinct two-layered tunica is always present in the apex of a floral bud (fig. 5, *A*; plate 2, *A*). Cells of the corpus that lie below *t-1-2* show periclinal and oblique divisions and form an area relatively as large as that of the leaf bud. Grégoire (1935), however, found no central corpus in the floral apices of *Magnolia* and *Ranunculus*. The type of cell divisions characteristic of each zone in the almond may be seen clearly in figure 5, *A*. Cytologically, the tunica cells are square and large in longitudinal section, containing a large nucleus characteristic of meristematic tissue. Epidermal cells are prominently cuticularized—more so than are the cells of the foliar apex.

In the corpus of the young bud, cells have the same uniform appearance for a depth of about 15 cells from the apex, where they are slightly larger and decidedly vacuolated and begin to form a rib meristem (fig. 5, *A*). Throughout the corpus, periclinal divisions (in relation to the apex) predominate, followed by oblique divisions; there are few if any anticlinal divisions, for apparently the oblique ones take care of the growth in width. In the lower part of the corpus very few cells contain tannin; it is noticeably lacking.

Apparently, therefore, elongation of the receptacle results from divisions in the corpus near the apex.

As the base of the bud is reached, the corpus tissue merges with the mature basal cells of the area that will become the peduncle. These cells, though not much larger than those of the corpus, are vacuolated, contain considerable amounts of what appears to be tannin, and are somewhat elongated in a horizontal direction. No evidence was noted of any of the mature tannin-filled cells' dividing. Tannin occurs in globular form.

Cataphylls are found almost immediately after initiation of the bud itself, and they cease to be produced only ten days before actual differentiation of floral organs takes place (fig. 4, *E*). As the apex is less advanced than that of the leaf bud in its initial stage, there are correspondingly fewer scales at a given time in the flower bud than in the other type during the early period, although this discrepancy is not noticed in mature buds.

Cells of the apex that give rise to bud-scale primordia contain much cytoplasm, for they stain more deeply than the surrounding tissues; these cells include both tunica and corpus. The formation of a cataphyll

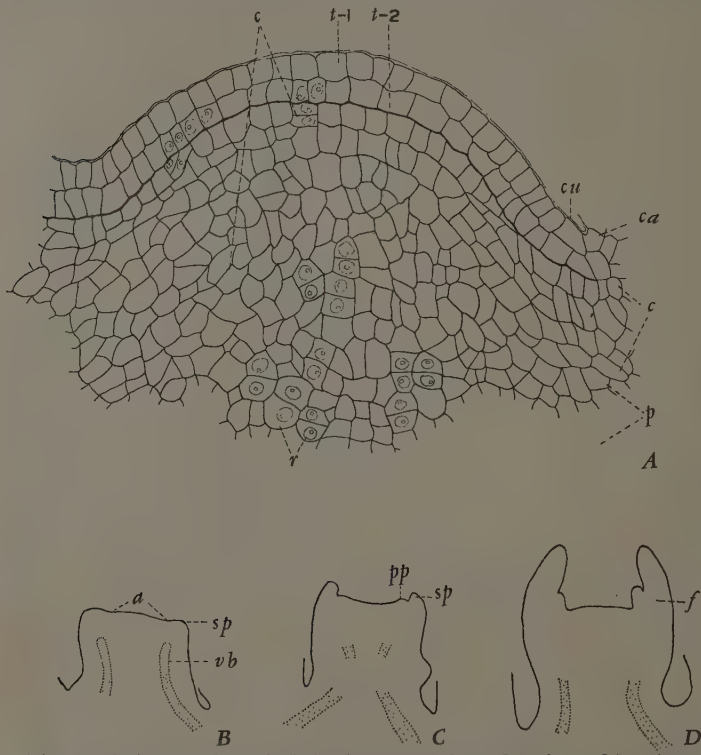

Fig. 5.—*A*, Apex of flower bud showing the two-layered tunica and location of corpus between the tunica and the rib meristem. The apex shows a prominent cuticle. *B*, Initiation of the sepal (stage 3) from the side of the apex at the end of the transition stage (stage 2). The vascular system is very near the primordium. *C*, Initiation of the petal (stage 4) from the sides of the apex between the sepal primordia. *D*, Formation of the floral tube by growth of the sepal and petal primordia. Details are: *a*, apex; *c*, corpus; *ca*, cataphyll; *cu*, cuticle; *ft*, floral tube; *p*, pith; *pp*, petal primoridum; *r*, rib meristem; *sp*, sepal primordium; *t-1*, *t-2*, first and second cell layers, respectively, of the tunica; *vb*, vascular bundle. (*A*, × 607; *B–D*, × 75.)

primordium resembles that of a foliage leaf: the tunica divides anticlinally, then periclinally; but its individuality soon becomes lost, although *t-1* always maintains its identity. There is evidence that the mature mesophyll of the cataphyll originates, at least in part, from the corpus.

Noticed in some of the cataphyll primordia is a definite subapical cell that cuts off cells obliquely and thus gives increase in height and width. The epidermal layer divides only anticlinally. Growth in length is accompanied by rapid cell divisions at and near the apex and base of the cataphyll; growth in width is caused by divisions in the corpus near the base. Cell enlargement follows the cell divisions, but is more evident nearer the base. The median procambial strand of the cataphyll usually differentiates early in the primordium.

Tannin appears early in the growth of the scale, being present in large amounts within two weeks after initiation. It appears first on the adaxial side in one or two cells of the subepidermal layer, and later appears in the epidermal layer. Subsequently it fills the same type of cells on the abaxial side. Finally, contiguous cells from the base upwards are filled, but none over the apex until the cataphyll approaches maturity. Tannin is in a solid mass in the scales, whereas it appears as globules in the corpus of the bud. In many cataphyll cells, tannin surrounds the nucleus, leaving a translucent area in each cell. Druses appear later than the tannin and always in cells other than those of the epidermis. Numerous hairs arise from the epidermal layer of the cataphyll.

At the end of this stage the number of cataphylls formed around the floral apex corresponds to the number (ranging from 10 to 18, with an average of 12.5) found on a mature bud at full bloom.

Stage 2, Transition from Vegetative to Floral Type of Apex.—A week before floral differentiation is noticeable in the formation of sepal primordia, there is an abrupt transition from the architecture typical of the apex which thus far has produced only vegetative structures, to the architecture typical of the apex which is to produce floral organs. This meristematic region is characterized first by becoming greatly elongated and broadened; and the dome-shaped form noted above in early stages is emphasized (fig. 4, *D* and *E*).

The striking histological feature accompanying this change in shape and size is the reduction in number of the tunica layers from two to one, so that the apex at the time of producing floral primordia is composed of a single-layered tunica and a massive corpus, larger than heretofore (fig. 4, *C* and *D;* plate 2). In all regions except the tunica, cell divisions are periclinal and oblique as well as anticlinal. Since cataphyll primordia have ceased to be produced, *"t-2"* is not dividing to form any such primordia, nor any floral primordia, as these appear several days later. The tunica, therefore, has lost its identity except for the one-celled surface layer, *t-1*. Thus, the cells of the corpus build up this apical region that becomes so typical of the floral-differentiation period.

Immediately before the production of floral primordia the apical surface undergoes a further change: it becomes flattened, and forms a broad plateau, slightly obovate in longitudinal section, but with its sides nearly vertical (fig. 4, *F*; plate 2, *B*). Its height has now been increased by growth in the toral region to 0.19 mm above the bases of the two innermost scale primordia. If an imaginary line is drawn between the bases of any two cataphylls, all the cells enclosed by that line and by *t-1* are derivatives of the corpus; all are similar in structure; and all are like the typical corpus of the preceding developmental stage. Those cells nearer the apex, however, take a deeper stain and are thus more meristematic than the basal ones, which are slightly vacuolated; yet they nevertheless retain meristematic properties. Few of these cells contain any tannin, and those only toward the base. The cuticle is thick and uniform over the epidermis.

A vascular system is well defined in the peduncle and extends upward into the lower portion of the receptacle (fig. 4, *F*; plate 2, *B*). Differentiation into xylem is beginning, as witnessed by the presence of spiral vessels.

The end of this transition period was September 1 in 1935 but came five days earlier in 1936. This stage is followed immediately by the production of floral organs in acropetal succession.

Stage 3, Initiation and Early Development of the Sepal.—The apex, preparatory to the initiation of floral organs, is thus greatly elongated in contrast to prior apices, either floral or vegetative, extending some 0.19 mm above the bases of the surrounding cataphylls. The tunica is reduced to a uniseriate surface layer, which covers a large corpus; and the internal tissue of the floral organs is derived, consequently, from this region, as the tunica maintains its identity only as a surface layer, maturing later into the epidermis (fig. 4, *F*; plate 2, *B*).

The first indication of a sepal primordium is the division in an anticlinal plane of the tunica cells located at the uppermost periphery of the apex. These divisions are accompanied in this area by periclinal ones in the subepidermal layer, the uppermost layer of the corpus; as a result, a slightly raised protuberance appears (fig. 5, *B*). The tunica cells maintain their original shape and do not elongate; in the vegetative primordia described above, they always elongate radially or anticlinally before cell division in the corpus. The meristematic region of this primordium extends basipetally to a greater extent than the cell divisions indicate, for the cells covering an area four rows deep show the characteristic deeper-staining qualities of such tissue. Because of the simultaneous initiation of the five sepal primordia, the apex is now slightly bowl-shaped, al-

though its center remains significantly convex. The surface of the entire summit is uniformly clothed with a heavy cuticle.

Cells of the corpus are meristematic, dividing anticlinally, periclinally, and tangentially; they extend downward some 12 cells until the vacuolated cells of the pith and cortex are reached. Boundaries of pith and cortex are indicated by elongated cells that form about 8 to 15 cells below the apex and 5 to 8 cells in from the surface layer; these cells constitute the procambium. The pith thus forms much of the receptacle, whereas the cortex remains small. The surface layer of the cortex apparently retains its meristematic nature, for the cells are square (viewed longitudinally) and contain a large nucleus surrounded by dense cytoplasm; the subepidermal layers are composed of vacuolated cells. The lower portion of this enlarged apex is the young peduncle, whereas it grades into toral tissue in the upper part of this massive axis tissue; differentiation has not proceeded far enough for exact structures to be demarcated.

In the basal portion of the axis and in the torus (but not in the corpus), tannin-filled cells occur, notably around the vascular system and epidermis; as tannin appears only in vacuolated cells, it is not present in the corpus. Some cells have only a few globules of tannin, whereas others are filled with one solid mass.

During the production of sepal primordia around the periphery of the apex, periclinal divisions continue in the remainder of the corpus, especially in the outer layer, which results in further growth in height of the entire axis. Continued periclinal divisions of the corpus within the initiated sepal give added length and, to a certain extent, added width. Basal cells of the sepal soon become vacuolated, and a procambial strand differentiates early in its ontogeny. In these respects the origin of the sepal closely resembles that of a foliage leaf.

The lower portion of this lateral floral structure differentiates later into a tube to which are attached the free floral parts (sepals, petals, and stamens); the result is a perigynous condition. Evidence from ontogeny in the almond apparently substantiates the view that this tube is floral, not receptacular. Hence the term *floral tube,* suggested by Jackson (1934) for other members of the Rosaceae, is used in place of *hypanthium, calyx tube,* or *receptacular tube,* which may imply toral origin.

When the calyx primordia reach a height of some 5 to 8 cells above the apex, petals are initiated; thus, petals are produced very shortly after the origin of the calyx.

Stage 4, Initiation and Early Development of the Petal.—Petal primordia arise alternately with the now-expanded sepal primordia (fig. 5, *C*). In the initiation of the petal the epidermal cells, after anticlinal

divisions, elongate anticlinally to almost twice the length of cells on either side; this elongation gives the slightly raised protuberance indicative of a primordium, although shortly the corpus divides periclinally. Divisions, mostly at right angles to the long axis, then proceed, as indicated for the sepal, to give height and width to the corolla primordia.

The meristematic apex between the petal primordia is flat (fig. 5, *C*), but soon becomes slightly rounded again (fig. 5, *D*). Further growth involves the floral tube, not the apex. During the growth of the petals the sepals elongate, show prominent procambial strands, and begin to arch over the apex slightly; growth is thus predominantly abaxial.

Stage 5, Initiation and Early Development of the Stamens.—Stamen initiation follows immediately upon the production of the corolla lobes. The floral apex is slightly dome-shaped, being raised some 3 to 4 cells, and is about 25 cells wide (fig. 6, *A*). The sepal and petal primordia have grown to form the visible beginnings of a floral tube, with the calyx growing inwardly over the other structures. It is upon the basal portion of the petal primordium, upon the structure which may be termed floral tube (although there is no line of demarcation between petal base and toral region), that the first evidence of a stamen appears. Thus the origin of the androecium is not from the apex, as in the other floral structures, but from the lowest point of the adaxial surface of the floral tube. As will be discussed later, this raised apical region is the first indication of the carpel; since such a structure as the carpel cannot give rise to other floral organs, the zone of growth and of initiation has shifted, in consequence, to a toral structure which produces the perigynous condition typical of the almond flower and which bears the matured stamens.

The stamen is initiated by anticlinal division in the surface layer, followed by periclinal and anticlinal divisions in the subepidermal row of cells of the floral tube. Growth of the newly divided tunica and corpus cells results in a slight protuberance, pushed out the width of a cell above the surface. Since there is no apical or subapical cell that may give rise to the inner tissues of the stamen, growth results from periclinal and anticlinal divisions of this central corpus tissue, although activity is greater in the subepidermal cells than in any others. In the apical layer of the corpus, adjacent cells may be dividing periclinally and anticlinally; this is the only cell row of the corpus that maintains its identity for any length of time. The meristematic region extends basipetally some 10 cells to the developing vascular system that leads from the petal.

As growth ensues, the cells of the tunica become much larger than those over the summit; yet they are not elongated. Covering the stamens is a definite cuticle like that over the other bud structures. As in all pre-

viously described primordia, the tunica divides only anticlinally; but the major part of the tissue comprising the stamen is cut off by successive

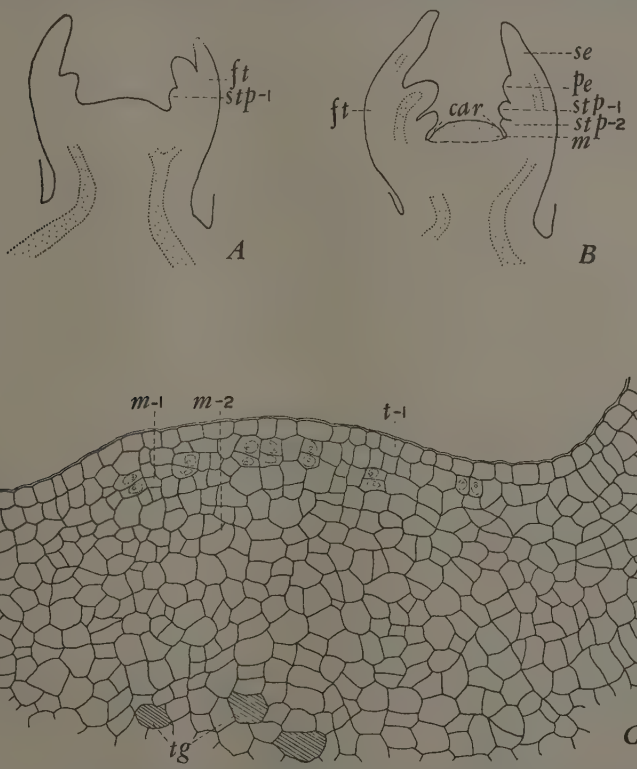

Fig. 6.—*A*, Flower bud showing initiation of the stamens of the outermost or abaxial whorl from the basal portion of the floral tube. *B*, Initiation of the carpel from the apex of the bud. The meristematic region is composed of two distinct types of cells, *m-1* and *m-2*. Procambial tissue is appearing in the petal primordium. *C*, Apex of the bud showing the initiation and emergence of the carpel from the entire width of the apex. Cell divisions, primarily in a periclinal plane, are localized in the uppermost four rows of cells. Cell division is also taking place in *m-2*, but to a lesser degree. The tunica is uniseriate. Details are: *car*, carpel primordium; *ft*, floral tube; *m*, highly meristematic zone; *m-1*, meristematic area of small cells containing a great deal of cytoplasm; *m-2*, meristematic area of large, vacuolated cells; *pe*, petal; *se*, sepal; *stp-1*, stamen primordium of first whorl; *stp-2*, stamen primordium of second whorl; *tg*, tannin globules; *t-1*, uniseriate tunica. (*A* and *B*, × 63; *C*, × 430.)

divisions, principally in the upper layer of the corpus. When the primordium is about 7 cells high, procambial cells appear 5 cells from the apex and at the base of the primordium.

The mature width of the filament is soon reached, for no periclinal divisions are observed. Very shortly all cells except about the six uppermost apical ones, as seen in longitudinal section, become vacuolated, including the surface layer. This condition does not, however, preclude cell division, for anticlinal divisions still proceed, giving increase in length. Tannin is soon found in the adaxial subepidermal layer and very near the apex of the stamen. The initiation of the stamen in each of the three whorls is similar.

The uppermost (abaxial) whorl of stamens arises first and opposite the calyx lobes (fig. 6, *A*). When this whorl is some 20 microns high, the second whorl is initiated, with every other filament opposite a petal primordium. Lastly, the short stamens of the innermost (adaxial) whorl arise. This sequence is in accord with the acropetal succession of floral organs and is substantiated, not only by an examination of serial sections showing this phase, but by macroscopic observation of the flower at the time of anthesis.

From observation of cell divisions in the calyx and corolla during this developmental period of the androecium, corpus tissue and its derivatives produce the mesophyll and the enclosed network of veins, although the mesophyll does not begin its differentiation until much later. Cell enlargement, accompanied by continued cell division, gives added growth in the floral tube, so that the sepals are beginning to touch overhead, resulting in an imbricated condition. Similar growth in the toral region (below the apex) produces height, with eventual delineation of a peduncle. The pith region is extremely large and contains much tannin. Outside the vascular system, in the cortex, cells are greatly vacuolated, including the surface layer.

Stage 6, Initiation of the Carpel.—The carpel originates on one side of the apex and begins by producing a slightly convex surface (fig. 6, *B*). As growth ensues, it becomes evident that this initial margin is the dorsal side of the carpel. The structure then forms in the shape of a horseshoe, with its two ventral (and open) edges or rims eventually uniting to form the adaxial suture. Thus this stage describes the changes occurring primarily in the dorsal portion of the apex, as nearly in a median plane as possible. Initiation of the carpel begins within three to five weeks (according to environmental conditions) after the transition of the vegetative apex to that characteristic of the floral apex.

The uniseriate tunica and upper subepidermal layers of the corpus of the apex show no localized meristematic activity during the formaton of petals. But as soon as cells have divided in the basal portion of the floral tube to form the stamens, active cell division is observed in the

apex—first indication of carpel initiation (fig. 2, *A*). As in all preceding structures, the anticlinal divisions of the tunica maintain its identity throughout development, finally producing the pubescence characteristic of the epicarp of the fruit. The apex is some 25 to 30 cells (175 to 190 microns) wide when the carpel primordium is formed, the entire apical surface being used for its production.

The meristem that initiates the carpel is composed of a definite layer of small cells, deeply stained, which extend from the uniseriate tunica to a depth of only 4 to 6 cells (*m-1* in fig. 6, *C;* fig. 5, *A*). These contain large nuclei and much cytoplasm. Cell division is predominantly periclinal throughout the entire uppermost layer of four cells, although with more divisions localized toward the center of the apex; as a result, the summit becomes slightly convex. At the time the second stamen whorl is initiated, the carpel primordium is some 4 to 7 cells high (fig. 6, *B*). Below these four to six rows of meristematic initials is a second region of meristematic cells, distinguished from the others by being larger and slightly vacuolated (*m-2* in fig. 6, *C;* fig. 5, *A*). These are immature and meristematic, are capable of extensive cell division, and extend basally some 4 to 8 cells, until the large and highly vacuolated cells of the pith are reached. Thus the corpus is composed of these two types of meristematic zones (*m* in fig. 6, *B,* and *m-1, m-2* in fig. 6, *C;* fig. 5, *A*) ; these areas maintain their identity in the developing carpel until exceedingly late.

As shown by serial sections, the apex on the side away from the initiating carpel is perfectly flat, with no meristematic tissue present in any part of the flat summit; carpel initiation is localized, therefore, on but one side of the apex. Below these meristematic areas are the more highly vacuolated and larger cells of the pith of the receptacle. In these cells a substance is found that apparently is tannin, for it stains a brilliant red and is comparable with the tannin of bud scales and leaves (fig. 6, *C;* fig. 5, *A*).

Stage 7, Development Prior to Union of the Carpel Edges.—As cell growth by division and enlargement proceeds on the dorsal side, cell division is initiated in two parallel lines running towards the ventral side; this is the beginning of the rims or edges that will finally unite to give the ventral suture. Cooper (1932) observed this same type of development in *Bougainvillea glabra.* Such growth results in a slope, as seen in longitudinal section, from the top of the dorsal side to the bottom of the ventral side (figs. 7, *A* and *B,* and 8, *A*). The dorsal margin tends to become vertical as growth ensues (fig. 7). Here, as in previous stages, the uniseriate tunica divides only by anticlinal divisions. The two meri-

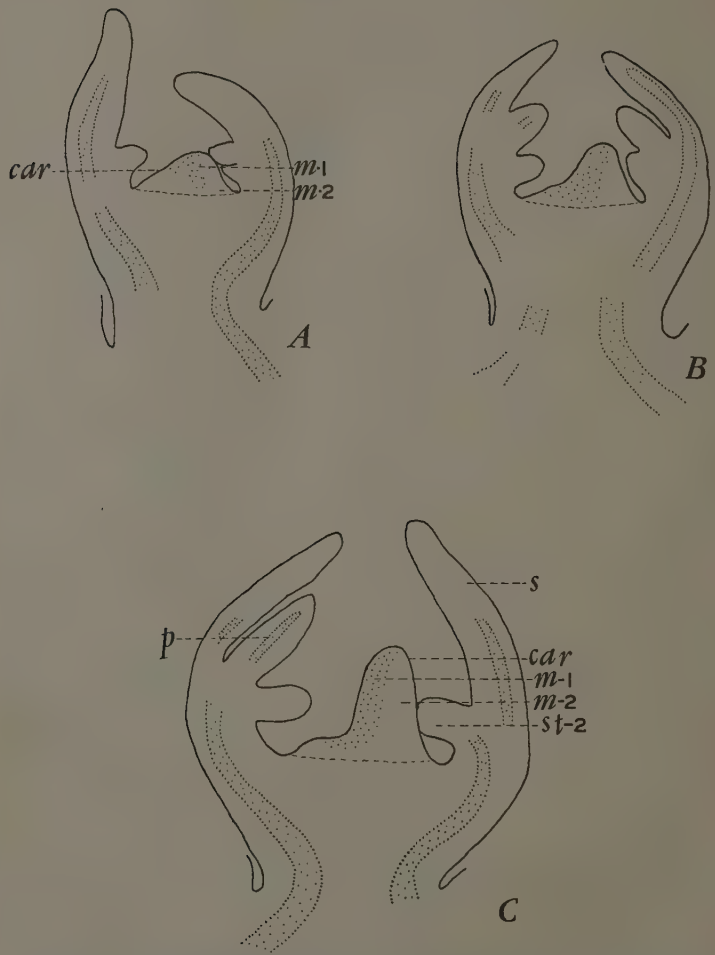

Fig. 7.—*A–C*, Longitudinal sections of the flower bud showing the emergence and successive stages in the growth of the carpel. The meristematic regions and persistent slope of the ventral edge (to the left in each drawing) are shown. Details are: *car*, carpel; *m-1*, meristematic area of small cells containing much cytoplasm; *m-2*, meristematic area of large, vacuolated cells; *p*, petal; *s*, sepal; *st-2*, stamen primordium of the second whorl. (All × 63.)

stematic zones noted in stage 6 are exceedingly noticeable at this point in development (fig. 8, *A;* plate 3, *B*).

The region noted by the symbol *m-1* in figure 7 is located primarily along the sloping ventral side, with a dip downwards toward the base of the carpel primordium. The center of this area is localized at the tip of the primordium (figs. 8, *A*, and 9; and plates 3, *B*, and 4); rapid periclinal, and to a certain degree anticlinal, divisions are characteristic of this region. The apex is covered with a heavy cuticle.

Viewing the carpel primordium transversely (fig. 8, *B*), its meristematic nature is obvious, particularly in the ventral edges, as would be expected from the location of these cells viewed in longitudinal section. The large, vacuolated cells (*m-2*) are about three to four times the size of the small cells near the periphery (*m-1*); this distinguishing feature is evident throughout development (figs. 7–9; plates 3 and 4). Many vacuolated cells show recent indications of cell division (fig. 9, *A*); the majority are periclinal, giving increase in height to the primordium. Cells typical of the pith are found several rows below the base of the carpel primordium. As is evident from the many divisions taking place in both types of meristem tissue, this region (the corpus) is responsible for producing the entire carpel. There is no indication of any procambium or any inorganic cell inclusions. The carpel shows its horseshoe-shaped structure almost throughout its height, as observed in transverse section (fig. 10, *J* and *K*).

If the carpel primordium is cut at right angles to the abaxial side, the nature of the groove that will form the locule may be observed, as in figure 10, *C*. The small meristematic cells characteristic of *m-1* are located at the tip of the primordium and around the groove; the vacuolated cells of *m-2* are around the periphery. The abaxial side is now practically vertical, for the serial section (not shown) next to figure 10, *A* shows no primordium at all, but only the flat surface of the receptacle directly against the raised floral tube on which are borne the stamens, petals, and sepals. At this time, the carpel is 185 microns wide—that is, from the vertical abaxial side to the flattened adaxial margin (fig. 10, *A–E*.

The ventral edges of the carpel have now developed (by October) considerably in height and, more significantly, in width, so that the open groove is beginning to be enclosed to form the locule. This growth in width is due primarily to cell division of the meristematic zone (*m-1*) which lines the central adaxial portion of the carpel. The abaxial tip of the carpel is beginning to assume the characteristic lobed shape of the stigma, for the edges have come together sufficiently at that point.

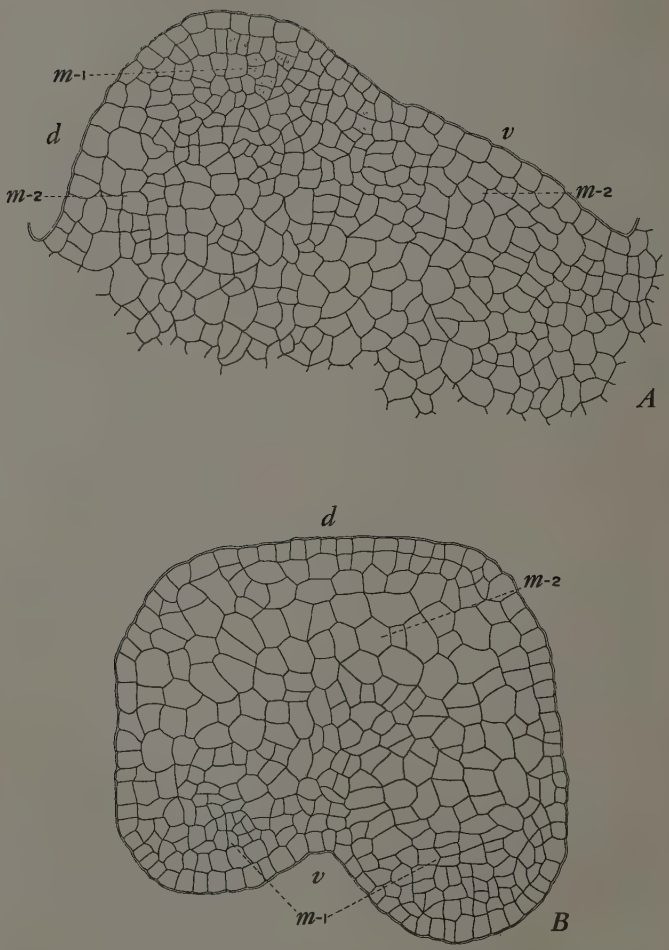

Fig. 8.—*A*, Longitudinal section of the carpel primordium showing the small cells characteristic of the apex and ventral side (*m-1*) and the large, vacuolated cells characteristic of the remainder of the primordium (*m-2*). *B*, Transverse section of the carpel primordium showing the highly meristematic area (*m-1*) of the two ventral margins, and the large cells of the dorsal side (*m-2*). The structures in this figure and in *A* were found in buds collected during the same week in October. Details are: *d*, dorsal side; *m-1*, meristematic area of small cells containing a great deal of cytoplasm; *m-2*, meristematic area of large, vacuolated cells; *v*, ventral side. (Both × 400.)

A procambium in the developing carpel is first noticeable about the middle of October. It appears in the central part of the primordium, on the abaxial side, and later connects with the vascular system of the torus. What seems to be tannin is found at the base of the primordium, in the receptacle (figs. 8 and 9, *B;* plate 4).

At the same stage of development as the carpel, the sepal, petal, and stamen whorls have developed considerably. The procambium is about to become differentiated. Tannin is abundant in the subepidermal layers and around the vascular strands in these organs; it is found in the epidermis of the sepal but not in the petal. Stamen attachment is already characteristic of that found in the mature flower (fig. 11, *A*), whereby there are three whorls of stamens alternating in a manner best shown by a floral diagram (fig. 1, *E*). The anther shows no differentiation into sporogenous tissue.

Stage 8, Development of the Carpel to Anthesis.—The ventral edges of the carpel come together to form the locule during the first week in November (two weeks after the preceding stage). The locule in longitudinal section is a narrow, elongated "slit" (fig. 10, *G;* plate 4, *B*). Union of the carpel edges to form the ventral suture does not involve cell growth nor true cell union across the suture; rather, the inner edges of the carpel mechanically touch each other more or less throughout the region (fig. 10, *I*). This loose condition of the ventral suture may last until the time of anthesis; the same condition has been observed in the peach by Ragland (1934). Soon after the formation of the ventral suture the locule enlarges considerably, preparatory to the production of the two ovules from placentae on the ventral margins (fig. 10, *G, H, I, L, M,* and *N*).

The carpel has developed considerably into definite regions— namely, ovary, style, and stigma (fig. 10, *H*). In the ovary a procambium has now arisen in each of the adaxial sides (fig. 10, *I*). The inner margins of the carpel, around the locule, maintain their characteristic meristematic activity (fig. 10, *I;* plate 4, *B*), as has been noted in much younger stages (fig. 7, *C*). This area gives rise to the ovule primordia. The remainder of the carpel shows the large, vacuolated cells of the other meristematic type (namely, *m-1* except for the vascular areas).

During this time (the latter part of November), there is no indication of any differentiation of the ovary wall into the three distinctive regions of the pericarp—epicarp, mesocarp, and endocarp (fig. 10, *M*). The earliest evidence of this differentiation comes in the latter part of January (two to three weeks before full bloom), when darkly stained cells form a "border" around the locule some 6 to 8 cells in from the epider-

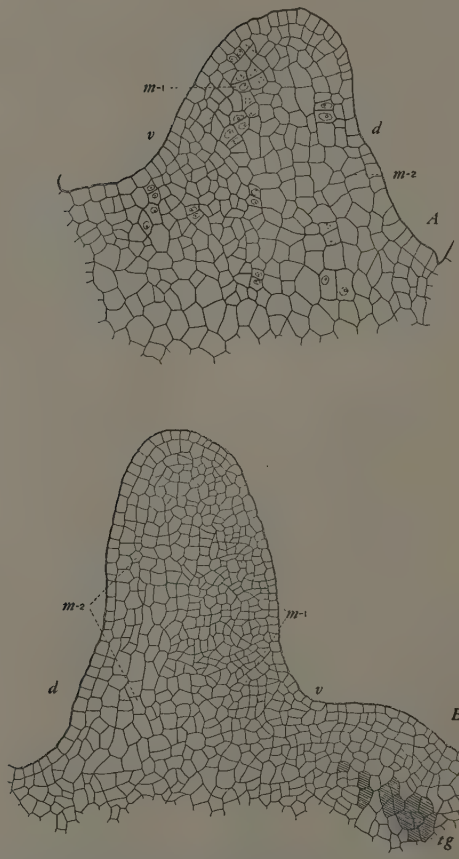

Fig. 9.—*A*, Longitudinal section of the developing carpel, showing cell divisions in both meristematic areas (*m-1* and *m-2*). The more highly meristematic region (*m-1*) is now found the full length of the ventral side. *B*, Longitudinal section of the carpel at a later date than that shown in *A*. The dorsal side is almost vertical, and is still composed of large vacuolated cells. Rapid cell division is occurring on the ventral side. Inclusions are found in the ventral basal cells. Details are: *d*, dorsal side; *m-1*, meristematic area of small cells containing much cytoplasm; *m-2*, meristematic area of large, vacuolated cells; *tg*, tannin globules; *v*, ventral side. (*A*, × 327; *B*, × 235.)

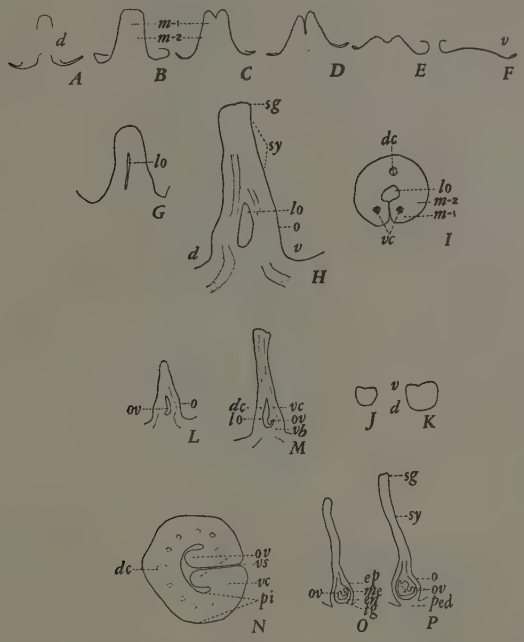

Fig. 10.—*A–F*, Consecutive longitudinal sections through the carpel (November 1, 1936) at right angles to the dorsal side, showing the groove and slope of the ventral margin and approximately in the same stage of development as the carpel in figures 7, *C*, and 9, *B*. The series represents 29 sections cut at 7 microns, of which numbers 1, 6, 12, 15, 22, and 29 are shown. *G*, Longitudinal section of the carpel in which the ventral margins have come together to produce the locule of the ovary. *H*, Longitudinal section of the carpel (November 26, 1936) showing its differentiation into stigma, style, and ovary. Dorsal and ventral bundles are present. *I*, Transverse section of the carpel (November 26, 1936) showing the enlarged locule and the ventral suture which is formed by the touching of its two margins. The ventral edges are highly meristematic. *J*, *K*, Transverse sections of the same carpel. *J* is 40 microns below the tip of the stigma and 80 microns above the receptacle; *K* is 48 microns below *J*. The cellular detail of *K* is shown in figure 8, *B*. *L*, Longitudinal section of the carpel (December 10, 1936) showing the initiation of the ovule from the basal ventral margin. *M*, Longitudinal section of the carpel (December 31, 1936) showing growth of the ovule. *N*, Transverse section of the carpel (February 8, 1937) showing the origin of the two ovules from the ventral margins, which are not yet closely united. *O*, *P*, Longitudinal sections of the carpel, showing the differentiation of the pericarp into endocarp and mesocarp. Growth has resulted in the orientation of the ovules at the stylar end of the locule. Two macrospores are present in the ovule in *O*. Details are: *d*, dorsal side; *dc*, dorsal carpellary bundle; *ep*, epicarp; *en*, endocarp; *lo*, locule; *m-1*, meristematic area of small cells; *m-2*, meristematic area of large, vacuolated cells; *me*, mesocarp; *o*, ovary; *ov*, ovule; *ped*, peduncle; *pi*, pericarp; *sg*, stigma; *sy*, style; *tg*, tannin globules; *v*, ventral side; *vb*, vascular bundle; *vc*, ventral carpellary bundle; *vs*, ventral suture. (*A–K* and *N*, × 34; *L* and *M*, × 12; *O* and *P*, × 3⅓.)

mis that bounds the locule (fig. 10, *O* and *P*). This area and the light-colored area toward the inside constitute the endocarp; the mesocarp extends from this region of darkly stained cells to the outermost three or more subepidermal cell layers composing the epicarp. These deeply stained cells apparently contain tannin, or precursors of the material composing the shell; they are found in cells between the primary bundles of the ovary wall (fig. 10, *N*). Most of the vascular bundles, therefore, are embedded in the endocarp (shell of the mature fruit) or in the "transition" tissue between endocarp and mesocarp (hull of the mature fruit). This latter condition is shown clearly at fruit maturity, when the hull particularly splits from the shell (fig. 11, *C*), leaving remnants of vascular tissue at random in the space thus formed. The ovary and style are exceedingly pubescent (fig. 11, *A*).

In the latter part of November the stigmatic lobes become well developed (fig. 10, *H*). During the last of December and all of January the style elongates so much that it is bent to one side by the pressure of the enveloping floral organs (fig. 10, *L*). In this stylar region the five or so cells next to the epidermis are large and vacuolated; the inner cells, found between the vacuolated ones as a central core, are small, full of cytoplasm, and deeply stained. In this latter region the vascular system develops, and what appears to be tannin is found.

The ovules are initiated about the second week in December, some eight weeks before full bloom; in the closely related peach (*Amygdalus Persica*), the initiation begins six weeks before full bloom (Ragland, 1934). The ovules originate as two small protuberances from the two highly meristematic adaxial margins, toward the base of the locule (fig. 10, *L* and *M*). Figure 10, *N*, shows their origin from the ventral margins; a space is still visible between the margins of the carpel. One ovule is usually somewhat higher than the other as regards attachment to the endocarp (fig. 10, *P*). After the production of these protuberances into the ovarian cavity, the ovules apparently remain dormant in this embryonic stage for several weeks until about January 1, when they mature rapidly by producing two integuments and sporogenous tissue. Two macrospores were visible in the macrosporangium in material collected January 31, 1936 (fig. 10, *O*). The gametophyte is formed and matured shortly thereafter, for the tree was in full bloom on February 15 of that year.

Growth in the base of the ovary wall orients the ovules so that their point of attachment to the pericarp is on the apical side of the locule, toward the style (figs. 10, *O* and *P*, and 11, *B*). The micropyle of each anatropous ovule points toward the style.

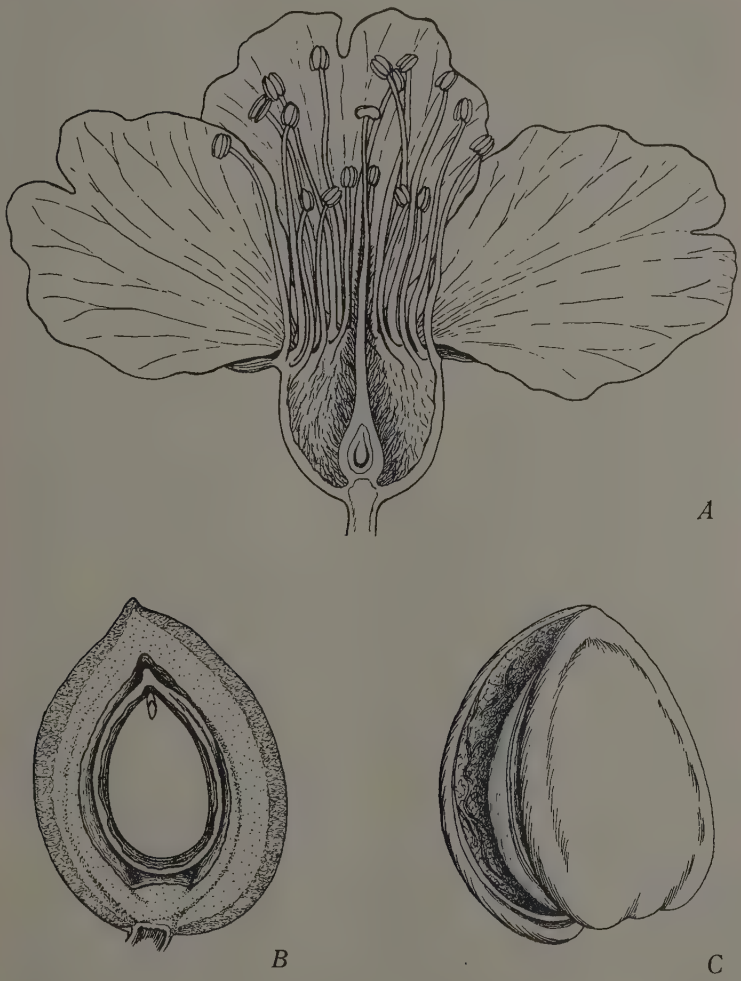

Fig. 11.—*A*, Longitudinal section of the perigynous flower of the almond at time of anthesis, showing the structure and location of the calyx, the corolla, the insertion and height of the stamen whorls, and the relation of the carpel to these other floral structures and to the floral tube. *B*, Median longitudinal section of a semimature fruit cut parallel to the sutures. This shows the differentiated pericarp, the locule, and the parts of the seed. *C*, Surface view of a mature fruit, showing the splitting of the mesocarp (hull) along the ventral suture which exposes the endocarp (shell). (*A*, ×7; *B*, ×1; *C*, ×1¼.)

Stamens continue their development so that by the latter part of November the sporogenous tissue has differentiated into pollen mother cells, and the primary parietal tissue into tapetal and connective tissue. Within two weeks tetrads appear, and by December 15 (1935) pollen grains are present in the anther sac; these are shed at the time of anthesis, two months later.

Ten months elapse between initiation of the carpel and its maturation at the time of harvest the following year; initiation took place from September 28 to October 1 in the years studied, and the fruit matures approximately the first few days in August.

DISCUSSION

In comparing histogenetically the vegetative and floral apices in the almond, it has been demonstrated that there are distinct differences in the initiation and early growth of foliage-leaf and floral organs, particularly with reference to the carpel. In the leaf bud the foliage leaf originates from a surface tissue, the tunica; the corpus does not enter into its formation in the early stages. In the flower bud the floral organs originate from tissues that had their origin deep in the bud and have "emerged" to the surface through progressive reduction of the tunica, from four layers in the leaf bud to two in the flower bud and then to one layer at the time of floral-organ formation. The question arises, therefore, should this progressive structural change be regarded as a "transformation" of a vegetative apex into a floral apex during its ontogeny? The idea that the plant forms but one type of apex from which arise foliage leaves, cataphylls, and floral organs, as maintained by Goebel (1933), is not supported by the present investigation (Foster, 1928; Sinnott, 1938; for physiological implications, see Loehwing, 1938).

In going from the vegetative to the reproductive phase in the almond flower bud, there is a definite "emergence" of tissue located at the surface of the apex. Accompanying this change is the reduction of the tunica from two layers to one layer, together with a modification of the architecture of the entire apical region. The tissues, therefore, and the direction of growth have been modified.

During the growth of the bud the cells of the corpus region divide in various planes, whereas tunica cells divide only anticlinally except at the time of initiation of primordia, and then only distal to the apex, next to the cataphyll or the foliage leaf bases as the case may be. Nowhere in either type of bud does the tunica in the median axial plane of the apex show periclinal cell division. Cataphylls, of both leaf and flower buds, and foliage leaves originate in the tunica at the periphery of the

apical meristematic area, although the corpus becomes involved *earlier* in the formation of the foliar structures of the flower bud than in that of the leaf bud. Formation of the floral organs involves corpus tissue exclusively (except for the surface layer), even though all floral structures except the carpel originate on the periphery of the apex, as noted for the foliar organs. The corpus, therefore, has an increasingly important rôle when a comparison is made of tissues involved in the formation of vegetative and reproductive organs.

In discussing the formation of the legume, J. McLean Thompson (1936*b*), together with Grégoire, holds that "the apex of the flower itself is the true primordium of the legume." Judging from the present investigation, the floral apex before carpel formation differs slightly from the apex at the time of carpel initiation. Before carpel formation the apex shows no localized meristematic zone and may be almost flat; but with initiation of this primordium the four apical layers of cells (fig. 6, *C*; fig. 5, *A*) become highly meristematic, giving rise to the protuberance that is the beginning of the carpel. To go back beyond this point: the apex of the flower bud during the formation of the other floral organs is histologically different from the apex at the time of carpel formation or carpel development. And before sepal initiation there is, of course, no trace of similarity. Although, at carpel initiation, the meristematic activity of the apex becomes more pronounced and the form of the apex slightly more dome-shaped, apparently the actual apex of the carpel has been present since the time of transition from vegetative to floral phase, though its development has been held in abeyance until the other organs have formed.

Newman (1936), in his work on the fruit of the *Acacia,* concluded that the legume was a lateral structure, not terminal as has generally been held. In his photomicrographs he indicates that cells "flow" away from the apex in the formation of the ovule-bearing organ, leaving behind a "residue" of the original apex. This residue, he found to be present at least until a stage immediately before the formation of ovule primordia, when it is then located at the base of the locule; the carpel wall has grown up and around it. Thompson (1936*b*) has raised the point that it is arbitrary where the "residue" of the original apex is indicated: the apex may or may not "remain" there, because meristems are not static.

According to the present study the entire apex becomes meristematic in producing the carpel. Similar stages in development are shown in *Amygdalus* (plate 3; figs. 6 and 7) and in *Acacia* (Newman, 1936, plate III, fig. 32); in figure 7, *A*, the label (*car*) indicating the carpel points directly to an area that Newman might call the residue of the

apex. In the almond, however, it is interpreted not as a residual portion of the apex, but as a stage of growth in the formation of the two adaxial edges of the carpel. Viewed transversely (fig. 8, *B*) and longitudinally at right angles to the dorsal side (fig. 10, *A*), the region in question is but one side of the ventral rim; the almond carpel starts its growth on the margin that is later noted to be the dorsal side, then extends immediately toward the ventral side by producing these two rims, all in the shape of a horseshoe. Thus the carpel may be interpreted as a terminal floral organ, in which no residual apex remains.

Grégoire (1936) remarks in criticizing Newman's study: "Nous croyons donc pouvoir conclure, sans aucune reserve, que l'origine du carpelle, dans les *Acacia,* est parfaitment terminale. Loin de contredire notre argumentation en faveur de l'autonomie morphologique du carpelle et du sommet floral dans les Angiospermes, les photographies de Newman ne font que la confirmer."

According to Grégoire (1931, 1938) the floral apex is entirely used up in the formation of the carpel; but A. Arber (1937) holds the contrary view. In the almond there is direct evidence that the apex is used up completely in the formation and development of the carpel. The entire apex enters directly into the initiation of the ovule-bearing structure, first on its future dorsal side, then with growth proceeding immediately toward the ventral side. The flower is truly a determinate structure, in contrast to the indeterminate leaf bud where, as far as was investigated, an apex was always present during production of cataphylls or leaves, throughout an entire year. Certain characteristics of the apex of the flower bud make it sporogenous in contrast to the purely vegetative axis of the leaf bud; growth of the sporogenous apex, at the time of carpel initiation, thus becomes limited in contrast to the unlimited growth of the leafy shoot, which continues to be meristematic throughout the life of the plant.

The apices of leaf and flower buds, at the same date, are of approximately the same relative size (fig. 2, *B*, and 4, *B*). A comparison of the apex of a flower bud in its vegetative (fig. 4, *B*) and reproductive state (figs. 4, *D* and *E*, and 5, *B*) shows a remarkable difference in the position and distribution of the meristematic tissue present. In comparing the limits of the various tissue areas in flower and leaf bud, one notes distinct differences. It seems reasonable to consider, as others do (Zimmermann, 1928; Newman, 1936), that the corpus region functions in increasing the volume of a vegetative apex, which is expressed mainly as increase in length. The tunica, by dividing anticlinally, produces increase only in surface. In the almond the surface layers diminish from

vegetative to floral condition, until at the time of floral initiation only a single discrete surface layer is maintained. The corpus, then, is found directly beneath the single surface layer and gives rise to all the *inner* tissues of the carpel. This fact supports the idea that the floral apex is of limited growth and that it leaves no residual axis behind.

In such meristematic regions as the embryo, Dixon (1936) and D'Arcy Thompson (1917) have shown that cell divisions do not control the growth of the parts. Actual observation, as indicated by these two men and by the present study, shows that growth precedes cell division, though the direction of growth may or may not control cell division. Dixon and D'Arcy Thompson conclude that the direction of growth controls cell division; but in the tunica layers of the almond this view does not appear to be correct.

In the initiation of any vegetative primordium the cells of *t-1* are the first to enlarge, elongating parallel to the longitudinal axis of the bud. These cells divide in such a direction as to give a daughter cell similar in length to the mother cell. Hence the succeeding cell division is not transverse to the elongation, as would be expected, possibly because of the mechanical rigidity of the elongated cell. The epidermal layer of the tunica is thus characterized solely by anticlinal divisions. In *t-2,* however, during the initiation of primordia, cell division is controlled by growth, as explained by Dixon (1936), for the division is periclinal in the elongated cell and transverse to this elongation. The apparent contradiction in relation between the direction of cell enlargement and the plane of cell division in the two outer tunica layers illustrates our lack of understanding of the mechanics of growth.

In reviewing the literature on the carpel, one finds two existing views concerning its interpretation: (1) the carpel is a modified or transformed foliage leaf (the classical Goethean Theory of Metamorphosis) and (2) the carpel is a distinct organ, in no way related to the foliage leaf except by analogy. As to the first interpretation, one main contribution that this theory has made to morphological studies through the years has been its use in correlating all the descriptions in floral morphology, especially in the field of taxonomy. Many taxonomists themselves hold to this viewpoint. Engler (1926), after more than fifty years of work, stated definitely that he adopted the foliar carpel interpretation for all angiosperms. Hutchinson (1926) clearly pronounced his own system to be a logical interpretation of the theory that the parts of an angiospermous flower are modified leaves.

From histological evidence Newman (1936) concludes that the carpel is homologous with the foliage leaf, although he did not compare the

floral apex to the vegetative apex of the two species of *Acacia* that he investigated. Grégoire (1935), in his earlier work, compared vegetative and floral apices taken, unfortunately, from plants of different genera (*Lonicera periclymenon* and *Ranunculus sceleratus*), and found characteristic differences between these apices. But in a more recent study of numerous species, in some of which he studied both vegetative and floral apices, Grégoire (1938) has considered the entire floral apex, in its production of all organs, as being distinctly different from the vegetative phase. According to him the apices of foliar and floral axes are "irreducible," and the tunica-corpus aspect is not applicable in the interpretation of the structure and growth of a floral apex. This concept agrees, in general, with the results of the present study. In the almond, however, to some extent, the presence of a uniseriate surface layer is suggestive of a tunica; also, the highly meristematic area found immediately below this layer implies a corpus as well as it does a mantle or hood, a conception which fits Grégoire's description of the floral apex. Grégoire naturally concludes that carpels do not represent modified leaves, but are organs without homology among vegetative organs.

In a similar vein, J. McLean Thompson has stated that sepals belong to the vegetative or sterile phase of the sporogenous axis (the flower), whereas the remaining portion pertains to the reproductive phase. According to him, furthermore, the carpel is not a leaf, but rather a phylloclade; as Clapham (1934) observes, to call it thus does not seem to help appreciably. Thompson (1935) also concludes that no carpels are involved in the organization of an inferior ovary—that it is acarpous.

Results of the present study indicate that the floral organs are all produced in a separate and distinct phase from that which produces foliar structures. Furthermore, just prior to formation of the characteristic floral apex, there is even a histological difference between apices that will become leaf buds and flower buds. The leaf arises from the tunica as a single primordium, continuing as a single linear structure and not as a "folded lamina" until a very late stage. The apocarpous almond carpel arises from the corpus as a primordium wth two lateral (ventral) protuberances, all in the form of a horseshoe, which will come together later to form the ventral suture and the ovarian cavity; this type of primordium is never observed in the formation of a foliar primordium. The almond carpel is not considered homologous with a foliage leaf. The mode of initiation and the areas from which leaf and carpel arise are distinctly and characteristically different (cf. fig. 3, *C*, and 9, *A*). This conception is directly opposed to that recently expressed by Just (Wilson and Just, 1939)—namely, "The origin of carpels on the

apex of the floral axis differs in no way from that of vegetative leaves, despite the fact that the growing point is frequently used up in their formation." These two organs may be analogous, but they are not homologous histogenetically in the almond. Likewise in a comparison of the mode and location of initiation of foliage leaf and floral organs other than carpel (sepals, petals, and stamens), all arise from a similar locus of the apex (on the periphery); but the tissue from which foliage and floral organs arise is distinctly different. Foliage leaves and cataphylls arise from a definite four-layered tunica. Sepals, petals, and stamens arise from superficial tissue; but, except perhaps the surface layer, this cannot be viewed as a tunica. Since homology implies a likeness in origin as well as in position (Cross, 1939), these two types of structures (vegetative and floral apices) are considered to be ontogenetically dissimilar.

Schmidt's (1924) tunica-corpus concept is evidently fundamental at the present time; let us hope that it rests upon sound premises. But if there is no such condition as tunica and corpus in floral apices, as Grégoire states, then our conception of apical structure must surely undergo revision. The almond may be a case intermediate between these two points of view.

The present study emphasizes Grégoire's contention that there is no histogenetic similarity between vegetative and reproductive apices. But, on the other hand, one cannot definitely state that no tunica and corpus are present in the floral apex of almond as in the vegetative apex.

Thus, certain evidence given here may aid in a true delineation of the nature of the carpel. Obviously, even the developmental history of an organ is not the "whole and sole 'way of salvation,'" as Goebel (1926) has stated; but, together with evidence from other sources, the histogenetic method is valuable in determining the present state of growth and development of structures, especially in their immature stages. In conclusion, it must be emphasized that the histogenetic approach to the problem of carpel morphology may be expected to yield more general results only when carried out along broad *comparative* lines. Such work should involve the detailed investigation and comparison of vegetative and floral apices in the same species in a wide series of angiospermous types.

SUMMARY

Marked histogenetic differences between leaf and flower buds in the almond (*Amygdalus communis* L., horticultural variety Nonpareil) apparently shed light upon the general problem of carpel morphology in the Angiospermae.

The apex of the leaf bud exhibits a four-layered tunica in contrast to the two tunica layers characteristic of the floral apex. The structure of the corpus is similar in both types of buds.

A week before the formation of the calyx, the form of the apex of the flower bud changes gradually from a broad dome to an elongated structure with a flat top and vertical flanks. During this change in external form the biseriate tunica is reduced to a single layer.

The foliage leaf primordium is derived from cells in the tunica, whereas all internal tissue of the floral organs originates from the corpus.

Cataphylls, foliage leaves, sepals, petals, and stamens originate as lateral members at the sides of the apex. In contrast, the entire apical region of the flower bud produces the carpel primordium.

Histogenetic evidence fails to support the classical interpretation that the carpel is homologous with a foliage leaf. It is concluded that from a developmental standpoint, the carpel is a distinct and unique organ among living angiospermous plants. Broad comparative histogenetic studies are urgent in order to test this interpretation.

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PLATES

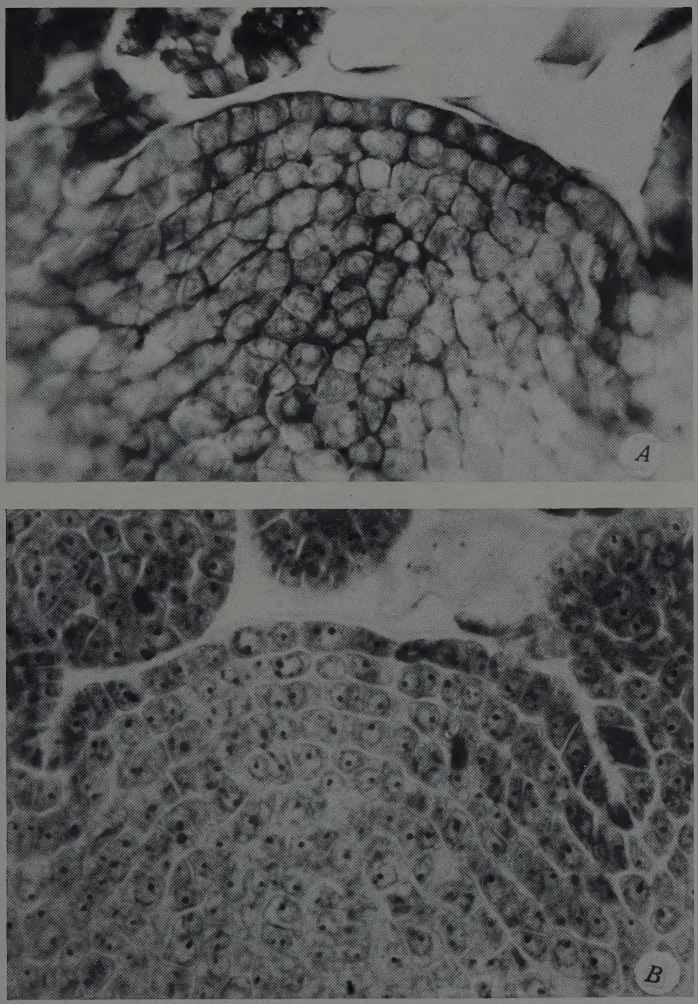


Plate 1.—Longitudinal sections of the apex of the leaf bud, showing the four tunica layers which surmount the corpus. *A*, Apex just before leaf arises. *B*, Apex showing the initiation of leaves on its periphery. Notice the elongated cells of the outer layer of the tunica (*t-1*), especially in the primordium at the left. (Both × 720.)

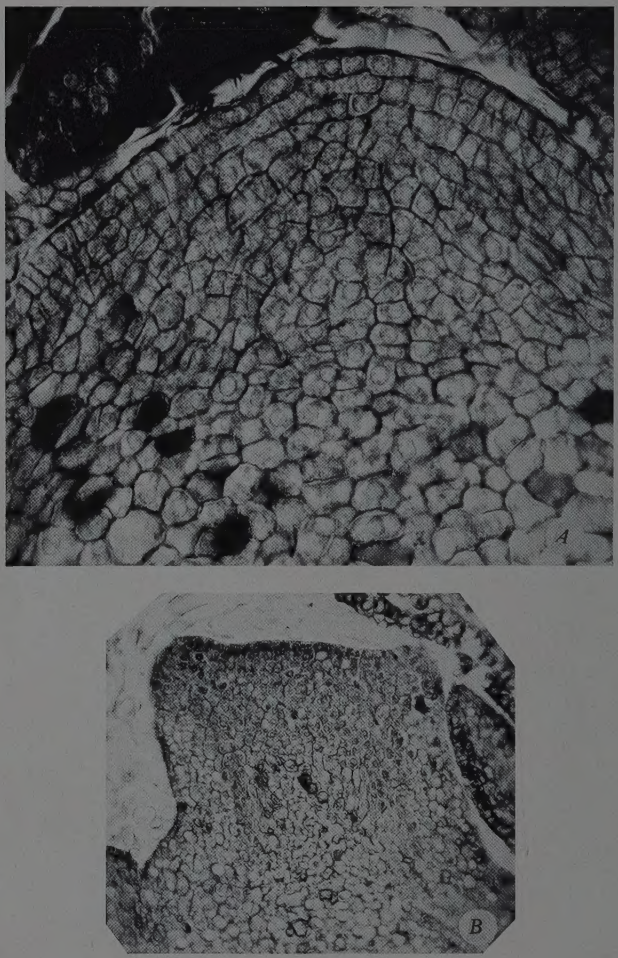


Plate 2.—Longitudinal sections of the flower bud. *A*, Apex showing the two layers of the tunica which surmount the corpus. Note the periclinal and oblique cell divisions in the first row of the corpus. Anticlinal divisions are characteristic of the tunica except during initiation of the cataphylls. *B*, The floral apex following transition from the purely vegetative apex in *A*. The tunica is now uniseriate. A procambial tissue is particularly noticeable on the left side of this massive axis. (*A*, × 565; *B*, × 275.)

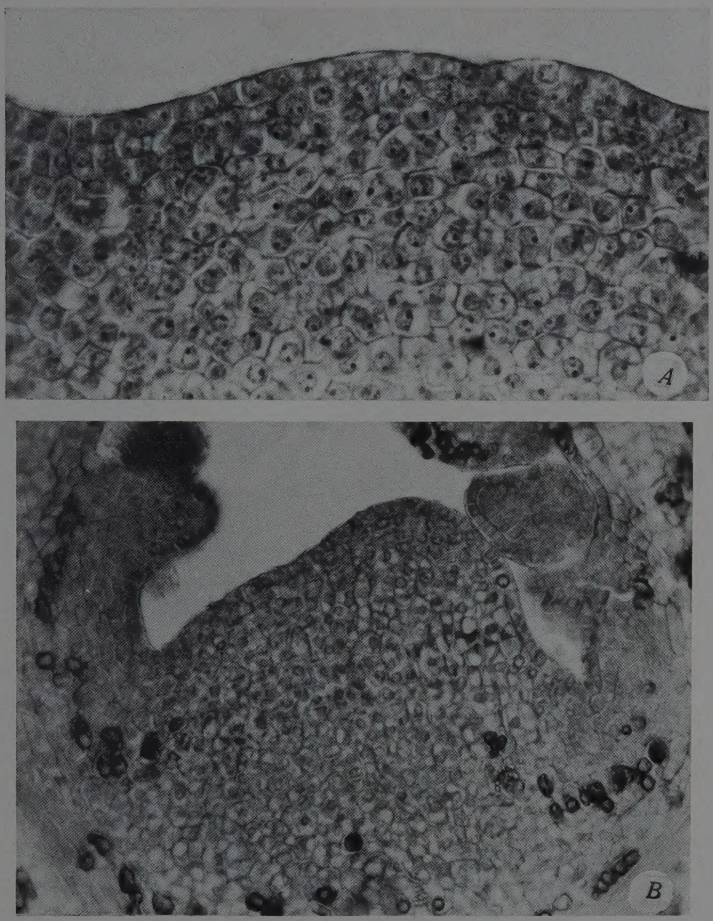
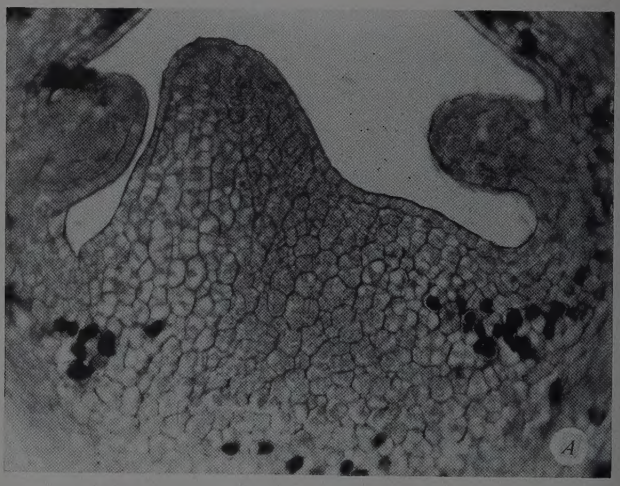


Plate 3.—Longitudinal sections of the flower bud, showing carpel initiation and development. *A*, Initiation of the carpel from the entire apex of the bud. Periclinal divisions are especially prevalent in the four layers of cells beneath the surface layer; below these layers are large, vacuolated cells which are also meristematic. At either side of the apex is the basal portion of the floral tube. *B*, The developing carpel, showing the rapid divisions in the tip and along the ventral margin. Large, vacuolated meristematic cells comprise the rest of this structure. The uniseriate tunica maintains its identity. The deeply stained cell inclusions are masses of tannin. (*A*, × 510; *B*, × 310).



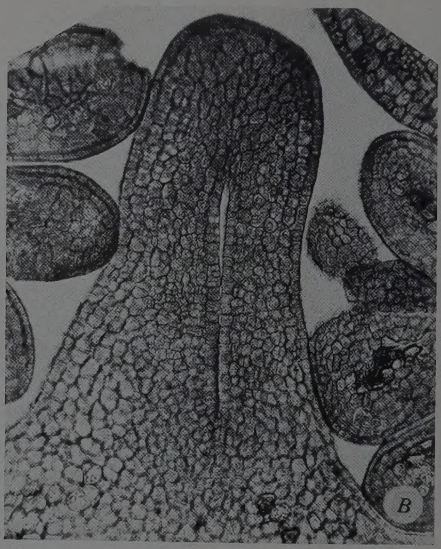


Plate 4.—Longitudinal sections of the carpel in more advanced stages. *A*, Carpel showing clearly the two different types of cells on the ventral (right) and dorsal sides of the structure; large vacuolated cells are characteristic of the dorsal side. Stamen primordia are found on either side arising from the floral tube. The deeply stained cell inclusions apparently are masses of tannin. *B*, The carpel showing the enclosed locule. Meristematic activity is centered around the locule. (*A*, × 275; *B*, × 185.)